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(54) Title: TIE-2, A NOVEL RECEPTOR TYROSINE KINASE			
(57) Abstract <p>The present invention relates to a novel receptor tyrosine kinase, herein referred to as <i>tie-2</i>, to nucleotide sequences and expression vectors encoding <i>tie-2</i>, and to methods of inhibiting <i>tie-2</i> activity. The invention also relates to other members of the <i>tie-2</i> receptor tyrosine kinase family. Genetically engineered host cells that express <i>tie-2</i> may be used to evaluate and screen drugs involved in <i>tie-2</i> activation and regulation. The invention relates to the use of such drugs as agonists or antagonists of <i>tie-2</i> activity.</p>			

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TIE-2, A NOVEL RECEPTOR TYROSINE KINASE1. INTRODUCTION

The present invention relates to a novel receptor
5 tyrosine kinase, herein referred to as tie-2, to
nucleotide sequences and expression vectors encoding
tie-2, and to methods of inhibiting tie-2 activity.
The invention also relates to other members of the
tie-2 receptor tyrosine kinase family. Genetically
10 engineered host cells that express tie-2 may be used
to evaluate and screen drugs involved in tie-2
activation and regulation. The invention relates to
the use of such drugs as agonists or antagonists of
tie-2 activity.

15

2. BACKGROUND

Receptor tyrosine kinases comprise a large and
evolutionarily conserved family of proteins comprised
of an extracellular ligand-binding domain, a
20 transmembrane domain and an intracellular tyrosine
kinase domain. Receptor tyrosine kinases are involved
in a variety of critical cellular processes such as
cellular differentiation and proliferation. The
binding of ligand to receptor tyrosine kinases induces
25 the formation of receptor dimers followed by
activation of receptor tyrosine kinase activity. This
in turn results in phosphorylation of a number of
intracellular substrates leading to a cascade of
events eventually resulting in cellular responses such
30 as changes in gene expression, cell morphology and
cell proliferation.

The formation and spreading of blood vessels, or
vasculogenesis and angiogenesis, respectively, play an
important role in a variety of physiological processes
35 such as embryonic development, wound healing and organ

regeneration. In particular, endothelial cell proliferation plays an important role during the development of the vascular system. In *in vitro* systems of angiogenesis, molecules that regulate tyrosine kinase activity have been found to modulate the invasion and tube formation of endothelial cells indicating that tyrosine phosphorylation plays a critical role in the regulation of endothelial cell proliferation and morphogenesis.

The cloning and characterization of two novel tyrosine kinases, expressed in endothelial cells, has recently been reported in the literature. Partanean et al. (1992, Mol. Cell. Biol. 12:1698-1707) identified a receptor tyrosine kinase, referred to as tie, which has 76% amino acid sequence homology with tie-2 in the cytoplasmic domain. The sequence homology between the two proteins diverges and is less pronounced in the extracellular and transmembrane domain (33% and 37% respectively). In addition, Dumont et al. (1992, Oncogene 7:1471-1480) reported the cloning and characterization of a partial cDNA clone encoding only the cytoplasmic domain of a novel receptor tyrosine kinase. The cloning of a full length representative of this particular clone, referred to as tek, has recently been reported and the deduced amino acid sequence of the tek tyrosine kinase receptor indicates broad homology with tie-2 (Ziegler et al., 1993, Oncogene 8:663-670).

The identification of novel tyrosine kinase receptors mediating physiological processes such as vasculogenesis and angiogenesis will lead to a more complete understanding of the molecular mechanisms controlling blood vessel formation. In addition, the identification of novel receptors involved in these processes will provide targets for development of therapeutic applications designed to treat disorders associated with aberrant blood vessel formation.

3. SUMMARY OF THE INVENTION

The present invention relates to a novel receptor tyrosine kinase receptor, herein referred to as *tie-2*, to nucleotide sequences and expression vectors encoding *tie-2*, and to methods of inhibiting *tie-2* activity. The invention is based, in part, on the isolation of a cDNA clone, from a brain capillary cDNA library, encoding the *tie-2* receptor tyrosine kinase.

The invention also relates to novel members of the *tie-2* family of receptor tyrosine kinases. More specifically, the invention relates to members of the *tie-2* family of receptor tyrosine kinases that are defined, herein, as those receptors demonstrating 80% homology at the amino acid level in substantial stretches of DNA sequence with *tie-2*. In addition, members of the *tie-2* family of receptor tyrosine kinases are defined as those receptors containing an intracellular tyrosine kinase domain and, in the extracellular region of the protein, EGF-like repeats flanked by a single amino terminal immunoglobulin (Ig) domain and a carboxyl-terminal triplet of fibronectin (Fn) type III domains.

Northern blot analysis and in situ hybridization indicates that *tie-2* is expressed in endothelial cell precursors (angioblasts) and in endothelial cells of the sprouting blood vessels throughout development and in all organs and tissues so far examined. Pharmaceutical reagents designed to modulate *tie-2* activity may be useful for treating diseases and/or processes associated with angiogenesis and vasculogenesis.

The invention relates to expression systems designed to produce *tie-2* receptor and/or cell lines expressing *tie-2* receptor. For example, engineered cell lines expressing *tie-2* on their surface may be advantageously used to identify *tie-2* ligands and to screen and identify agonist and antagonist of the *tie-*

2 receptor. Additionally, expression of soluble recombinant *tie-2* may be used to generate antibodies against specific epitopes of the *tie-2* protein and/or to screen peptide libraries for molecules that bind *tie-2*.

5

4. BRIEF DESCRIPTION OF THE FIGURES

Fig. 1A, 1B and 1C. Nucleotide Sequence of murine *tie-2*. The AUG START codon and AGA STOP codon are underlined.

10

Fig. 2A. Deduced amino acid sequence and structure of *tie-2*. Amino acid sequence of *tie-2* in single letter code. The potential signal sequence cleavage site is indicated by an arrowhead. Black dots mark the two cysteine residues, possible involved in sulfhydryl bonding of the immunoglobulin domain. The three EGF-like repeats are boxed. The three fibronectin type III domains are underlined. The transmembrane region is given in bold face letters. The tyrosine kinase domain is indicated by shaded boxes. The RGD triplet is marked by an asterisk.

15

20

Fig. 2B. Schematic diagram of the structure of *tie-2*. Ig, immunoglobulin domain; EGF, EGF-like repeats; FN, fibronectin type III domains; Kinase, tyrosine kinase domain; KI, kinase insertion.

25

Fig. 3. Amino acid sequence comparison of *tek*, *TEK* and *tie* with *tie-2*. Amino acid residues identical to *tie-2* are represented by (-). Gaps are indicated by (.).

30

Fig. 4. Northern analysis of *tie-2* expression in brain capillaries and total brain tissue from postnatal day 4 (P4) mice. A single transcript of approximately 4.7 kb, highly enriched in the capillary fraction was detected.

35

Fig. 5. *tie-2* expression during brain development. Sagittal sections of adult brain (A,B), postnatal day 4 brain (C,D,G,H) and embryonic day 12.5

brain (E,F) were hybridized with a *tie-2* probe. Bright-field (A,C,E) and corresponding dark-field micrographs (B,D,F) Arrowheads indicate expression in capillaries and arrows indicate hybridization signals over meningeal blood vessels. Higher magnification of a capillary sprout at the dorsal surface of a postnatal day 4 telencephalon (G). Higher magnification of a capillary in a deeper layer of the brain (H). No other than the vascular elements of the brain were found to synthesize *tie-2* mRNA. LM, leptomeninges; LV, lateral ventricle; TE, telencephalon; (A,B,C,D,E,F) bar represents 110 μm ; (G,H) bar represents 10 μm ;

Fig. 6. Colocalization of *tie-2* expression with immunostaining for PECAM (CD31). Adjacent section of E12.5 embryos were hybridize with a *tie-2* probe (A) or stained with antibody recognizing PECAM (CD31) (B). Arrowheads indicate expression of *tie-2* in the endothelial cell layer of a medium-sized blood vessel from the head region. Bar represents 10 μm .

Fig. 7. *Tie-2* expression in organs and tissues of E 12.5 embryos and colocalization with immunostaining for PECAM (CD31). Adjacent sections of E 12.5 embryos were stained with an antibody recognizing PECAM (CD31) (A,C,E,G) or hybridized with a *tie-2* probe (B,D,F,H). The expression pattern of *tie-2* was found to be identical to the staining pattern fort PECAM (CD31). TE, telencephalon; LV, lateral ventricle; CP, choroid plexus of the lateral ventricle; DA, dorsal aorta; SC, spinal cord; EN, endocardium; MY, myocardium; BR, bronchus; SO, somite; Bar represents 110 μm .

Fig. 8. *Tie-2* expression at embryonic day 8.5. Transverse section of an E 8.5 embryo and adjacent yolk sac. Hybridization signals were detectable in the endocardium, dorsal aorta, cardinal vein and the mesodermal (inner layer) of the yolk sac (A,B).

Arrowheads indicate hybridization signal over the marginal cells of an advanced stage blood island (C,D). Note the absence of hybridization signals in the neuroectoderm. CV, cardinal vein (head vein); BI, blood island; DA, dorsal aorta; EN, endocardial tissue; TE, telencephalon; YS, yolk sac. Bar, represents 50 μ m.

5. DETAILED DESCRIPTION

The present invention relates to a novel receptor tyrosine kinases referred to herein as tie-2. The invention is based, in part, on the isolation of a cDNA clone encoding the tie-2 receptor tyrosine kinase from a brain capillary cDNA library. The invention also relates to novel members of the tie-2 receptor tyrosine kinase family as defined herein.

Results from Northern Blot analysis and *in situ* hybridization indicates that tie-2 is expressed specifically in the endothelial cell lineage. Tie-2 transcripts could be detected in endothelial cell precursors (angioblasts) and in endothelial cells of sprouting blood vessels throughout development and in all organs and tissues so far examined.

The invention relates to expression of the tie-2 receptor and/or cell lines that express the tie-2 receptor which may be used to screen for antibodies, peptides, or other ligands that act as agonists or antagonists of the tie-2 receptor. For example, anti-tie-2 antibodies may be used to inhibit tie-2 function. Alternatively, screening of peptide libraries with recombinantly expressed soluble tie-2 protein or cell lines expressing tie-2 protein may be useful for identification of therapeutic molecules that function by regulating the biological activity of tie-2.

For clarity of discussion, the invention is described in the subsections below by way of example

for the murine *tie-2*. However, the principles may be analogously applied to clone and express the *tie-2* receptor of other species including humans.

5.1. THE TIE-2 CODING SEQUENCE

5 The nucleotide coding sequence and deduced amino acid sequence of the *tie-2* gene is depicted in Figures 1A, 1B, 1C (SEQ. ID NO: __) and Figure 2A (SEQ. ID NO: __), respectively. In accordance with the invention, any nucleotide sequence which encodes the amino acid sequence of the *tie-2* gene product can
10 be used to generate recombinant molecules which direct the expression of *tie-2*. In additional embodiments of the invention, nucleotide sequences which selectively hybridize to the *tie-2* nucleotide sequence shown in FIG. 1 (SEQ ID NO: __) may also be used to express
15 gene products with *tie-2* activity. Hereinafter all such variants of the *tie-2* nucleotide sequence will be referred to as the *tie-2* DNA sequence.

 In a specific embodiment described herein, the
20 *tie-2* gene was isolated by performing a polymerase chain reaction (PCR) in combination with two degenerate oligonucleotide primer pools that were designed from conserved protein regions of tyrosine kinases. As a template cDNA synthesized by reverse
25 transcription of purified mRNA from capillaries of pooled P4-P8 mice brains was used. Gel-purified reaction products of the expected size were radiolabelled and used directly to screen a cDNA library constructed from the remainder of the
30 capillary mRNA. Sequence analysis of the inserts from positive phages revealed that 13 of the cDNAs were derived from one mRNA species. The longest of these 13 clones, a fragment of 4640 bp, was sequenced completely. It contained a long open reading frame
35 encoding a protein of 1123 amino acid residues.

Within the extracellular part, the FASTA program detected homologies to proteins including TAN-1 (Ellisen et al., 1991 Cell 66:649-661), Xotch (Coffman et al., 1990), Laminin (Sasaki et al., 1988 J. Biol. Chem. 263:16536-16544). Delta (Vässin et al., 1987 EMBO J. 6:3431-3440) and Perlecan (Noonan et al., 1991 J. Biol. Chem. 266:22939-22947) (around 30% sequence identity). The structural basis for these homologies are three EGF-like repeats in the center of the extracellular portion of tie-2 (Fig. 2A). EGF-like repeats consist of 30 to 40 amino acid residues often found in the extracellular parts of membrane-bound proteins or secreted proteins (Davies, 1990 New Biol 5:410-419). A common feature of these domains are six conserved cysteine residues, which are known to be involved in disulfide bonds. The three EGF domains in tie-2 contain two additional cysteine residues at the carboxyl-terminal end of each repeat.

The central EGF-like repeats are flanked by two different structural motifs, a single amino-terminal immunoglobulin (Ig) domain and a carboxyl-terminal triplet of fibronectin (FN) type III domains. Although the Ig domain does not exhibit clear sequence similarities to other known proteins (except for tie and TEK), the Ig domain has the conserved features of a C2-set domain including typical residues surrounding the first cysteine residue and the canonical GxYxC found at the second cysteine residue (Williams and Barclay, 1988 Ann. Rev. Immunol. 6:381-405).

The three fibronectin type III (FN III) domains are most closely related to FN III repeats present in the protein-tyrosine phosphatases Delta (Krueger et al., 1990 EMBO J. 9:3241-3252), LAR (Streuli et al., 1988 J. Exp. Med. 168:1553-1562) and DLAR (Streuli et al., 1989 Proc. Natl. Acad. Sci USA 86:8698-8702) and to FN III repeats of the axonal glycoprotein TAG-1 (Furley et al., 1990 Cell 61:157-170) (around 20%

sequence identity). These domains have been described as units of approximately 90 amino acids containing hydrophobic residues at characteristic positions. In addition, a total of nine potential N-glycosylation sites are located in the extracellular part of *tie-2*.

5 The invention also relates to *tie-2* genes isolated from other species, including humans. Members of the *tie-2* family are defined herein as those receptors containing an intracellular tyrosine kinase domain, and, in the extracellular domain, EGF-like
10 repeats flanked by two different structural motifs, a single amino-terminal immunoglobulin (Ig) domain and a carboxyl-terminal triplet of fibronectin (FN) type III domains. Such receptors may demonstrate about 80%
15 homology at the nucleotide level, and even 90% homology at the amino acid level in substantial stretches of DNA sequence.

 To isolate the *tie-2* gene from other species a bacteriophage cDNA library may be screened, under conditions of reduced stringency, using a
20 radioactively labeled fragment of the murine *tie-2* clone. Alternatively the murine *tie-2* sequence can be used to design degenerate or fully degenerate oligonucleotide probes which can be used as PCR probes or to screen bacteriophage cDNA libraries. A polymerase chain reaction (PCR) based strategy may be used
25 to clone, for example, the human *tie-2*. Two pools of degenerate oligonucleotides, corresponding to conserved motifs within the murine *tie-2* may be designed to serve as primers in a PCR reaction. Conserved motifs may include the tyrosine kinase
30 domain, the EGF-like repeats, the immunoglobulin (Ig) domain or the fibronectin (Fn) type III domains. The template for the reaction is cDNA obtained by reverse transcription of mRNA prepared from cell lines or
35 tissue known to express human *tie-2* such as blood capillaries or endothelial cells. The PCR product may

be subcloned and sequenced to insure that the amplified sequences represent the *tie-2* sequences. The PCR fragment may be used to isolate a full length *tie-2* cDNA clone by radioactively labeling the amplified fragment and screening a bacteriophage cDNA library. Alternatively, the labeled fragment may be used to screen a genomic library. For a review of cloning strategies which may be used, see e.g., Maniatis, 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, (Green Publishing Associates and Wiley Interscience, N.Y.)

Northern Blot analysis and *in situ* hybridization indicated that *tie-2* was expressed in blood vessels during brain angiogenesis. Figure 4 demonstrates the presence of a 4.7 Mb mRNA highly enriched in the capillary fraction. A single RNA species could also be detected in organs like the brain, kidney and heart.

In situ hybridization experiments indicated that *tie-2* mRNA was exclusively synthesized in the vasculature of adult brain, P4 brain, E12.5 brain E8.5 neuroectoderm (FIG. 5A-H). In P4 brain, *tie-2* expression was detected in capillaries that are about to invade the neural tissue as well as in vessels that have already reached deep layers of neuroectoderm. In addition to capillaries, meningeal blood vessels and the choroid plexus were also found to synthesize *tie-2* mRNA at comparable levels (FIGS. 5, 7B). At E12.5 the overall expression pattern was virtually identical to that observed at P4, although the density of labelled structures in E12.5 brain was reduced (Fig. 5C,0). This observation correlates with the less extensive vascularization of the embryonic brain (Bär, 1980 Adv. Anat. Embryol Cell Biol. 59:1-62). No hybridization signals were observed in the neuroectoderm of E8.5

embryos, because at that stage this tissue is still avascular (Fig. 8). In the adult brain, tie-2 expression was detectable, although it seemed to be reduced when compared to postnatal or embryonic stages. Hybridization signals persisted over larger vessels especially those of the meninges (Fig. 5A,B).
5 The synthesis of tie-2 mRNA in smaller vessels and capillaries was barely detectable.

To identify the cell type, expressing tie-2 mRNA, *in situ* hybridization and immunohistochemistry was performed. The pattern of tie-2 expression was
10 compared with the staining of cells with a monoclonal antibody against the endothelial cell-specific adhesion molecule PECAM (CD31). Figure 6 demonstrates the overlap between cells stained by the PECAM
15 antibody and those cells labeling with a tie-2 probe. Figure 7 presents a survey on the coexpression of tie-2 and PECAM in several organs and tissues and it is clear from the results that tie-2 is coexpressed with PECAM in several organs and tissues.

The possible role of tie-2 in early stages of
20 vascular development was investigated by *in situ* hybridization studies with E8.5 sections. The probe detected tie-2 mRNA in the mesodermal component of the yolk sac (FIG. 8). This finding is of particular
25 interest, because it is in the mesodermal component of the yolk sac that the first signs of blood vessel development are evident. Clusters of mesenchymal cells form the so-called blood islands. At the margin of these aggregates cells, the so-called angioblasts,
30 adopt an endothelial-like phenotype, whereas in the center cells differentiate into embryonic hemoblasts. Fig. 8 demonstrates that the peripheral angioblasts synthesize high levels of tie-2 mRNA.

Within the embryo proper, expression was seen in
35 the anlagen of the vascular system, e.g. in the developing endocardium, the dorsal aortae and the

cardinal veins (Fig. 8A,B). It is likely that these signals stem from intraembryonic endothelial cell precursors, which are present in these structures at that stage, tie-2 expression could also be detected in the allantois and in blood vessels of the maternal decidua.

5

5.2. EXPRESSION OF TIE-2 RECEPTOR
AND GENERATION OF CELL LINES THAT
EXPRESS TIE-2

In accordance with the invention, tie-2
10 nucleotide sequences which encode tie-2, peptide
fragments of tie-2, tie-2 fusion proteins or
functional equivalents thereof may be used to generate
recombinant DNA molecules that direct the expression
of tie-2 protein or a functionally equivalent thereof,
15 in appropriate host cells. Alternatively, nucleotide
sequences which hybridize to portions of the tie-2
sequence may also be used in nucleic acid
hybridization assays, Southern and Northern blot
analyses, etc.

20 Due to the inherent degeneracy of the genetic
code, other DNA sequences which encode substantially
the same or a functionally equivalent amino acid
sequence, may be used in the practice of the invention
for the cloning and expression of the tie-2 protein.
25 Such DNA sequences include those which are capable of
hybridizing to the murine tie-2 sequence under
selective conditions.

Altered DNA sequences which may be used in accor-
dance with the invention include deletions, additions
30 or substitutions of different nucleotide residues
resulting in a sequence that encodes the same or a
functionally equivalent gene product. These
alterations would in all likelihood be in regions of
tie-2 that do not constitute functionally conserved
35 regions. In contrast, alterations, such as deletions,

additions or substitutions of nucleotide residues in functionally conserved *tie-2* regions would be expected to result in a nonfunctional *tie-2* receptor. The gene product itself may contain deletions, additions or substitutions of amino acid residues within the *tie-2* sequence, which result in a silent change thus producing a functionally equivalent *tie-2*. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipatic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine. The DNA sequences of the invention may be engineered in order to alter the *tie-2* coding sequence for a variety of ends including but not limited to alterations which modify processing and expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, e.g. site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, phosphorylation, etc. For example, in certain expression systems such as yeast, host cells may over glycosylate the gene product. When using such expression systems it may be preferable to alter the *tie-2* coding sequence to eliminate any N-linked glycosylation site.

In another embodiment of the invention, the *tie-2* or a modified *tie-2* sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries it may be useful to encode a chimeric *tie-2* protein expressing a heterologous epitope that is recognized by a

commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the tie-2 sequence and the heterologous protein sequence, so that the tie-2 may be cleaved away from the heterologous moiety.

5 In an alternate embodiment of the invention, the coding sequence of tie-2 could be synthesized in whole or in part, using chemical methods well known in the art. See, for example, Caruthers, et al., 1980, Nuc. Acids Res. Symp. Ser. 7:215-233; Crea and Horn, 180, Nuc. Acids Res. 9(10):2331; Matteucci and Caruthers,
10 1980, Tetrahedron Letters 21:719; and Chow and Kempe, 1981, Nuc. Acids Res. 9(12):2807-2817. Alternatively, the protein itself could be produced using chemical methods to synthesize the tie-2 amino acid sequence in whole or in part. For example, peptides can be
15 synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography. (e.g., see Creighton, 1983, Proteins Structures And Molecular Principles, W.H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman and
20 Co., N.Y., pp. 34-49.

25 In order to express a biologically active tie-2, the nucleotide sequence coding for tie-2, or a functional equivalent, is inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and
30 translation of the inserted coding sequence. The tie-2 gene products as well as host cells or cell lines transfected or transformed with recombinant tie-2 expression vectors can be used for a variety of purposes. These include but are not limited to
35 generating antibodies (i.e., monoclonal or polyclonal)

that bind to the receptor, including those that competitively inhibit binding of tie-2 ligand and "neutralize" activity of tie-2 and the screening and selection of drugs that act via the tie-2 receptor; etc.

5

5.2.1: EXPRESSION SYSTEMS

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the tie-2 coding sequence and appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y.

A variety of host-expression vector systems may be utilized to express the tie-2 coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the tie-2 coding sequence; yeast transformed with recombinant yeast expression vectors containing the tie-2 coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the tie-2 coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the tie-2 coding sequence; or animal cell systems.

35

The expression elements of these systems vary in their strength and specificities. Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used in the expression vector. For example, when
5 cloning in bacterial systems, inducible promoters such as pL of bacteriophage λ , plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when cloning in insect cell systems, promoters such as the baculovirus polyhedrin promoter may be used; when
10 cloning in plant cell systems, promoters derived from the genome of plant cells (e.g., heat shock promoters; the promoter for the small subunit of RUBISCO; the promoter for the chlorophyll a/b binding protein) or from plant viruses (e.g., the 35S RNA promoter of
15 CaMV; the coat protein promoter of TMV) may be used; when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia
20 virus 7.5K promoter) may be used; when generating cell lines that contain multiple copies of the tie-2 DNA, SV40-, BPV- and EBV-based vectors may be used with an appropriate selectable marker.

In bacterial systems a number of expression
25 vectors may be advantageously selected depending upon the use intended for the tie-2 expressed. For example, when large quantities of tie-2 are to be produced for the generation of antibodies or to screen
30 peptide libraries, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include but are not limited to the E. coli expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791),
35 in which the tie-2 coding sequence may be ligated into the vector in frame with the lacZ coding region so

that a hybrid tie-2/lacZ protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In
5 general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are
10 designed to include thrombin or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety.

In yeast, a number of vectors containing constitutive or inducible promoters may be used. For
15 a review see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant et al., 1987, Expression and Secretion Vectors for Yeast, in
20 Methods in Enzymology, Eds. Wu & Grossman, 1987, Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; and Bitter, 1987, Heterologous Gene Expression in Yeast,
25 Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and The Molecular Biology of the Yeast Saccharomyces, 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I
and II.

In cases where plant expression vectors are used, the expression of the tie-2 coding sequence may be
30 driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Brisson et al., 1984, Nature 310:511-514), or the coat protein promoter of TMV (Takamatsu et al., 1987, EMBO J. 6:307-311) may be
35 used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al., 1984, EMBO J.

3:1671-1680; Broglie et al., 1984, Science 224:838-843); or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley et al., 1986, Mol. Cell. Biol. 6:559-565) may be used. These constructs can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, microinjection, electroporation, etc. For reviews of such techniques see, for example, Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp. 421-463; and Grierson & Corey, 1988, Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9.

An alternative expression system which could be used to express tie-2 is an insect system. In one such system, Autographa californica nuclear polyhydrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The tie-2 coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the tie-2 coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect Spodoptera frugiperda cells in which the inserted gene is expressed. (E.g., see Smith et al., 1983, J. Virol. 46:584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the tie-2 coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus

genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing *tie-2* in infected hosts. (E.g., See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. (USA) 81:3655-3659). Alternatively, the vaccinia 7.5K promoter may be used. (See, e.g., Mackett et al., 1982, Proc. Natl. Acad. Sci. (USA) 79:7415-7419; Mackett et al., 1984, J. Virol. 49:857-864; Panicali et al., 1982, Proc. Natl. Acad. Sci. 79:4927-4931).

Specific initiation signals may also be required for efficient translation of inserted *tie-2* coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where the entire *tie-2* gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the *tie-2* coding sequence is inserted, exogenous translational control signals, including the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the *tie-2* coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:516-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage)

of protein products may be important for the function of the protein. The presence of nine consensus N-glycosylation sites in the *tie-2* extracellular domain indicate that proper modification may be important for *tie-2* function. Different host cells have characteristic and specific mechanisms for the post-transla-

Appropriate cells lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, WI38, etc.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the *tie-2* may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with the *tie-2* DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the *tie-2* on the cell surface. Such engineered cell lines are

particularly useful in screening for drugs that affect tie-2.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk⁻, hgp^rt⁻ or ap^rt⁻ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981), Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hyg^r, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30:147) genes. Recently, additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, 1988, Proc. Natl. Acad. Sci. USA 85:8047); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue L., 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.).

5.2.2. IDENTIFICATION OF TRANSFECTANTS OR TRANSFORMANTS THAT EXPRESS THE TIE-2

The host cells which contain the coding sequence and which express the biologically active gene product

may be identified by at least four general approaches;
(a) DNA-DNA or DNA-RNA hybridization; (b) the presence
or absence of "marker" gene functions; (c) assessing
the level of transcription as measured by the expres-
sion of *tie-2* mRNA transcripts in the host cell; and
(d) detection of the gene product as measured by
immunoassay or by its biological activity.

In the first approach, the presence of the *tie-2*
coding sequence inserted in the expression vector can
be detected by DNA-DNA or DNA-RNA hybridization using
probes comprising nucleotide sequences that are
homologous to the *tie-2* coding sequence, respectively,
or portions or derivatives thereof.

In the second approach, the recombinant expres-
sion vector/host system can be identified and selected
based upon the presence or absence of certain "marker"
gene functions (e.g., thymidine kinase activity,
resistance to antibiotics, resistance to methotrexate,
transformation phenotype, occlusion body formation in
baculovirus, etc.). For example, if the *tie-2* coding
sequence is inserted within a marker gene sequence of
the vector, recombinants containing the *tie-2* coding
sequence can be identified by the absence of the
marker gene function. Alternatively, a marker gene
can be placed in tandem with the *tie-2* sequence under
the control of the same or different promoter used to
control the expression of the *tie-2* coding sequence.
Expression of the marker in response to induction or
selection indicates expression of the *tie-2* coding
sequence.

In the third approach, transcriptional activity
for the *tie-2* coding region can be assessed by
hybridization assays. For example, RNA can be
isolated and analyzed by Northern blot using a probe
homologous to the *tie-2* coding sequence or particular
portions thereof. Alternatively, total nucleic acids

of the host cell may be extracted and assayed for hybridization to such probes.

In the fourth approach, the expression of the tie-2 protein product can be assessed immunologically, for example by Western blots, immunoassays such as radioimmuno-precipitation, enzyme-linked immunoassays and the like.

5.3. USES OF THE *TIE*-2 RECEPTOR AND ENGINEERED CELL LINES

The uses of the tie-2 receptor and engineered cell lines, described in the subsections below, may be employed equally well for the tie-2 family of receptor tyrosine kinases.

In an embodiment of the invention, engineered cell lines which express the entire tie-2 coding region or its ligand binding domain may be utilized to screen and identify the natural ligand and/or ligand antagonists as well as agonists. Synthetic compounds, natural products, and other sources of potentially biologically active materials can be screened in a number of ways.

5.3.1 IDENTIFICATION OF *TIE*-2 LIGAND USING ENGINEERED CELL LINES

Engineered cell lines that express the tie-2 receptor, may be advantageously used to identify and characterize the natural tie-2 ligand(s). For example, a genomic or cDNA library may be transfected into the engineered cell lines. Any cell that expresses and secretes the tie-2 ligand, due to the transfer of a DNA clone capable of encoding the tie-2 ligand, will stimulate the biological activity of the recombinantly expressed the tie-2 receptor. The resulting autocrine loop will lead to preferential proliferation of cells expressing tie-2 ligand and the

resulting cell colonies may be used to isolate the DNA encoding the *tie-2* ligand.

The engineered cell lines may also be used to assay tissue or cell extracts for their ability to activate *tie-2* receptor tyrosine kinase activity.

5 Activation of *tie-2* tyrosine kinase activity may be assayed using a variety of methods. For example, in cells overexpressing the *tie-2* receptor, overall incorporation of labeled PO_4 into the cell may be measured after contacting cells with extracts.

10 Alternatively, the *tie-2* protein may be immunoprecipitated following stimulation with extracts, using anti-*tie-2* antibodies, followed by Western blot analysis using anti-tyrosine phosphate antibodies to determine whether the immunoprecipitated *tie-2* has been tyrosine phosphorylated.

15 Once an extract is identified which contains *tie-2* ligand, various procedures and techniques known in the art which include but are not limited to chromatography (e.g., reverse phase liquid, gel permeation, liquid exchange, ion exchange, size
20 exclusion, affinity chromatography), centrifugation, electrophoretic procedures, differential solubility, or other standard techniques may be used to purify *tie-2* ligand.

25 In a preferred embodiment, affinity chromatography using recombinantly expressed *tie-2* covalently attached to a column matrix may be used to purify *tie-2* ligand. Alternatively, a recombinantly expressed hybrid protein comprised of the *tie-2*
30 extracellular domain fused to the immunoglobulin protein A binding domain may be used to purify *tie-2* ligand. The fusion protein may be used to prepare a column matrix over which cell extracts may be added, or, the fusion protein may be used to
35 immunoprecipitate the *tie-2* ligand from cell extracts.

Once purified, the tie-2 ligand may be subjected to microsequencing, using techniques routinely used by those skilled in the art to determine the amino acid sequence of a protein. If the tie-2 ligand molecule is blocked at the amino terminus, the protein may be chemically cleaved or partially enzymatically digested to yield peptide fragments that may be purified and sequenced.

A mixture of degenerate oligonucleotide probes may be designed using the information derived from the protein sequencing of the purified tie-2 ligand. The oligonucleotides may be labeled and used directly to screen a cDNA library for clones containing inserts with sequence homology to the oligonucleotide sequences. Alternatively, the oligonucleotides may be used as primers in a polymerase chain reaction. The amplified DNA fragment may be labeled and used to screen a library for isolation of full length clones.

5.3.2. SCREENING OF PEPTIDE LIBRARY WITH TIE-2 PROTEIN OR ENGINEERED CELL LINES

Random peptide libraries consisting of all possible combinations of amino acids attached to a solid phase support may be used to identify peptides that are able to bind to the ligand binding site of a given receptor or other functional domains of a receptor such as kinase domains (Lam, K.S. et al., 1991, Nature 354: 82-84). The screening of peptide libraries may have therapeutic value in the discovery of pharmaceutical agents that act to inhibit the biological activity of receptors through their interactions with the given receptor.

Identification of molecules that are able to bind to the tie-2 may be accomplished by screening a peptide library with recombinant soluble tie-2 protein. Methods for expression and purification of

tie-2 are described in Section 5.2.1 and may be used to express recombinant full length *tie-2* or fragments of *tie-2* depending on the functional domains of interest. For example, the kinase and extracellular ligand binding domains of *tie-2* may be separately expressed and used to screen peptide libraries.

5 To identify and isolate the peptide/solid phase support that interacts and forms a complex with *tie-2*, it is necessary to label or "tag" the *tie-2* molecule. The *tie-2* protein may be conjugated to enzymes such as
10 alkaline phosphatase or horseradish peroxidase or to other reagents such as fluorescent labels which may include fluorescein isothiocyanate (FITC), phycoerythrin (PE) or rhodamine. Conjugation of any given label, to *tie-2*, may be performed using
15 techniques that are routine in the art.

Alternatively, *tie-2* expression vectors may be engineered to express a chimeric *tie-2* protein containing an epitope for which a commercially available antibody exist. The epitope specific
20 antibody may be tagged using methods well known in the art including labeling with enzymes, fluorescent dyes or colored or magnetic beads.

To screen a peptide library the "tagged" *tie-2* conjugate is incubated with the random peptide library
25 for 30 minutes to one hour at 22°C to allow complex formation between *tie-2* and peptide species within the library. The library is then washed to remove any unbound *tie-2* protein. If *tie-2* has been conjugated to alkaline phosphatase or horseradish peroxidase the
30 whole library is poured into a petri dish containing substrates for either alkaline phosphatase or peroxidase, for example, 5-bromo-4-chloro-3-indoyl phosphate (BCIP) or 3,3',4,4"-diaminobenzidine (DAB), respectively. After incubating for several minutes,
35 the peptide/solid phase-*tie-2* complex changes color, and can be easily identified and isolated physically

under a dissecting microscope with a micromanipulator. If a fluorescent tagged tie-2 molecule has been used, complexes may be isolated by fluorescent activated sorting. If a chimeric tie-2 protein expressing a heterologous epitope has been used, detection of the peptide/tie-2 complex may be accomplished by using a
5 labeled epitope specific antibody. Once isolated, the identity of the peptide attached to the solid phase support may be determined by peptide sequencing.

In addition to using soluble tie-2 molecules, in another embodiment, it is possible to detect peptides
10 that bind to cell surface receptors using intact cells. The use of intact cells is preferred for use with receptors that are multi-subunits or labile or with receptors that require the lipid domain of the cell membrane to be functional. Methods for
15 generating cell lines expressing tie-2 are described in Sections 5.2.1. and 5.2.2. The cells used in this technique may be either live or fixed cells. The cells will be incubated with the random peptide
20 library and will bind to certain peptides in the library to form a "rosette" between the target cells and the relevant solid phase support/peptide. The rosette can thereafter be isolated by differential centrifugation or removed physically under a
25 dissecting microscope.

As an alternative to whole cell assays for membrane bound receptors or receptors that require the lipid domain of the cell membrane to be functional, the receptor molecules can be reconstituted into
30 liposomes where label or "tag" can be attached.

5.3.3. ANTIBODY PRODUCTION AND SCREENING

Various procedures known in the art may be used for the production of antibodies to epitopes of the
35 recombinantly produced tie-2 receptor. Such

antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression library. Neutralizing antibodies i.e., those which compete for the ligand binding site of the receptor are especially preferred for diagnostics and therapeutics.

Monoclonal antibodies that bind tie-2 may be radioactively labeled allowing one to follow their location and distribution in the body after injection. Radioactivity tagged antibodies may be used as a non-invasive diagnostic tool for imaging *de novo* cells expressing tie-2.

Immunotoxins may also be designed which target cytotoxic agents to specific sites in the body. For example, high affinity tie-2 specific monoclonal antibodies may be covalently complexed to bacterial or plant toxins, such as diphtheria toxin, abrin or ricin. A general method of preparation of antibody/hybrid molecules may involve use of thiol-crosslinking reagents such as SPDP, which attack the primary amino groups on the antibody and by disulfide exchange, attach the toxin to the antibody. The hybrid antibodies may be used to specifically eliminate tie-2 expressing cells.

For the production of antibodies, various host animals may be immunized by injection with the tie-2 protein including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacilli Calmette-Guerin) and Corynebacterium parvum.

Monoclonal antibodies to tie-2 may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein, (Nature, 1975, 256:495-497), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today, 4:72; Cote et al., 1983, Proc. Natl. Acad. Sci., 80:2026-2030) and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce tie-2-specific single chain antibodies.

Antibody fragments which contain specific binding sites of tie-2 may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab'), fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab'), fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity to tie-2.

5.4. USES OF TIE-2 CODING SEQUENCE

The tie-2 coding sequence may be used for diagnostic purposes for detection of tie-2 expression. Included in the scope of the invention are oligoribonucleotide sequences, that include antisense RNA and DNA molecules and ribozymes that function to inhibit translation of tie-2. In addition, mutated forms of tie-2, having a dominant negative effect, may be expressed in targeted cell populations to inhibit the activity of endogenously expressed tie-2.

5.4.1. USE OF TIE-2 CODING SEQUENCE IN DIAGNOSTICS AND THERAPEUTICS

The tie-2 DNA may have a number of uses for the diagnosis of diseases resulting from aberrant expression of tie-2. For example, the tie-2 DNA sequence may be used in hybridization assays of biopsies or autopsies to diagnose abnormalities of tie-2 expression; e.g., Southern or Northern analysis, including *in situ* hybridization assays.

Also within the scope of the invention are oligoribonucleotide sequences, that include anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of tie-2 mRNA. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between -10 and +10 regions of the tie-2 nucleotide sequence, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. Within the scope of the invention are engineered

hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of tie-2 RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of

flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

5

5.4.2. USE OF DOMINANT NEGATIVE
TIE-2 MUTANTS IN GENE THERAPY

Receptor dimerization induced by ligands, is thought to provide an allosteric regulatory signal that functions to couple ligand binding to stimulation of kinase activity. Defective receptors can function as dominant negative mutations by suppressing the activation and response of normal receptors by formation of unproductive heterodimers. Therefore, defective receptors can be engineered into recombinant viral vectors and used in gene therapy in individuals that inappropriately express tie-2.

In an embodiment of the invention, mutant forms of the tie-2 molecule having a dominant negative effect may be identified by expression in selected cells. Deletion or missense mutants of tie-2 that retain the ability to form dimers with wild type tie-2 protein but cannot function in signal transduction may be used to inhibit the biological activity of the endogenous wild type tie-2. For example, the cytoplasmic kinase domain of tie-2 may be deleted resulting in a truncated tie-2 molecule that is still able to undergo dimerization with endogenous wild type receptors but unable to transduce a signal.

Recombinant viruses may be engineered to express dominant negative forms of tie-2 which may be used to inhibit the activity of the wild type endogenous tie-2. These viruses may be used therapeutically for treatment of diseases resulting from aberrant expression or activity of tie-2, such as cancers.

35

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of recombinant *tie-2* into the targeted cell population. Methods which are well known to those skilled in the art can be used to construct those recombinant viral vectors containing *tie-2* coding sequence. See, for example, the techniques described in Maniatis et al., 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y. Alternatively, recombinant *tie-2* molecules can be reconstituted into liposomes for delivery to target cells.

6. EXAMPLES: CLONING AND CHARACTER-
IZATION OF THE *TIE-2*
RECEPTOR TYROSINE KINASE

6.1. MATERIALS AND METHODS

6.1.1. ANIMALS AND TISSUES

Balb/c mice were mated overnight and the morning of vaginal plug detection was defined as 0.5 day of gestation. Organs were removed, frozen immediately in liquid nitrogen and stored at -80°C. Capillary fragments from pooled P4-P8 mice brains were prepared according to Risau et al. (1990 J. Cell Biol. 110:1757-1766). For *in situ* hybridization and immunohistochemistry, whole embryos or organs were fixed for 12 hours in freshly prepared 4% paraformaldehyde in PBS at 4°C, rinsed for 24-48 hours in 0.5 M sucrose at PBS at 4°C, embedded in TissueTek (Miles) and stored frozen at -80°C.

6.1.2. RNA EXTRACTION AND ANALYSIS

Total cytoplasmic RNA was isolated according to the method of Chomczynski and Sacchi (1987 Anal. Biochem. 162:156-159). Enrichment for poly(A)⁺ containing fractions was achieved by oligo(dT) chromatography using push columns (Stratagene).
 5 Aliquots of poly(A)⁺ RNA were electrophoresed in agarose gels containing 0.66 M formaldehyde and transferred to Zeta-Probe membrane (Bio Rad) in 20x SSPE. Hybridizations were performed overnight in 0.5
 10 M sodium phosphate buffer, 5% SDS, 1% BSA, pH 7.5 at 68°C with 1x10⁶ cts/minute/ml of probe, which had been labelled with ³²P-dCTP according to the protocol of a random-primed DNA-labeling kit (Boehringer Mannheim). Membranes were washed under high-stringency conditions
 15 at 68°C in 0.1XSSPE. 0.5% SDS and autoradiographed at -80°C on Fuji films. Poly(A)⁺ RNA from brain capillary fragments was isolated using a QuickPrep Micro mRNA purification kit from Pharmacia.

6.1.3. PCR AND cDNA CLONING

20 Capillary poly(A)⁺ RNA was reverse transcribed and aliquots of the cDNA were used as templates in PCR reactions. The following oligonucleotides were used as primers: 5'-CAC/TCGIGAC/TC/TTIGCIGCIA/CG-3', 5'-
 25 AC/TICCIAA/CIC/GA/TCCAIACA/CTC-3' (I stands for inosine) in addition to the following premiss described by Wilks et al. (1989, DPNAS Proc. Natl. Acad. Sci. USA 86:1603-1607):

5'-CGGATCCACAGNGACCT-3' and
 C TT

30 3'-CTGCAGACCAGGAAACCTTAAGG-5'
 A C T

The amplification products were separated in acrylamide gels. Fragments of the expected size were purified, labeled and used as probes to screen a
 35 random hexanucleotide-primed cDNA library constructed

with a Time Saver cDNA Synthesis Kit (Pharmacia).
cDNA fragments were subcloned into Bluescript vectors
(Stratagene). Sequencing was done by using nested
oligonucleotide primers in combination with a 373 DNA
Sequencer (Applied Biosystems) and with the Convention
Sequenase System (USB).

5

6.1.4. IN SITU HYBRIDIZATION

Preparation of tissue sections and *in situ*
hybridizations with single-stranded DNA probes or
single-stranded RNA probes was performed as described
by Schnürch and Risau (1991, Development 111:1143-
1154). The probe for all hybridization experiments
was derived from a 1179 bp DNA fragment encoding a
portion of the putative tie-2 protein from amino acid
419 to amino acid 812.

10

15

6.1.5. IMMUNOHISTOCHEMISTRY

Embedded embryos and whole organs were sectioned
on a Leitz cryostat 8 μ m sections were mounted on
organosilane-treated slides, dried overnight under
vacuum and stored desiccated at -80°C. Sections were
brought to room temperature, rehydrated in PBS for 5
minutes and incubated in 0.1% H₂O₂ in methanol for 15
minutes. After washing three times in PBS for 5
minutes each, nonspecific antibody binding was blocked
by application of 20% normal goat serum in PBS for 20
minutes. Sections were washed, incubated with a rat
monoclonal antibody against mouse PECAM for one hour,
washed again and then incubated with a viotinylated
gout anti-rat IgG (Dianova) for one hours. Color
development was performed with a Vectastain ABC Kit
(Vector Laboratories) according to the vendor's
protocol. Sections were slightly counterstained with
toluene blue, dehydrated and mounted.

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6.2. RESULTS

6.2.1. cDNA CLONING AND STRUCTURE OF THE TIE-2 PROTEIN

The approach for the isolation of receptor tyrosine kinases expressed in endothelial cells of sprouting blood vessels involved the following steps.

5 First, mRNA was purified from capillaries of pooled P4-P8 mice brains. A portion of the mRNA was reverse transcribed and used in PCR reactions with degenerate primers deduced from conserved protein regions of tyrosine kinases. Gel-purified reaction products of
10 the expected size were radiolabelled and used directly as hybridization probes to screen a cDNA library constructed from the remainder of the capillary mRNA.

Partial sequencing of the inserts from positive phages revealed that 13 of the cDNAs were derived from
15 one mRNA species. The longest of these 13 cDNAs, a fragment of 4640 bp, was sequenced completely (FIG. 1A, 1B, 1C). It contained a long open reading frame encoding a protein of 1123 amino acid residues (FIG. 2A). This deduced polypeptide has all features of a
20 receptor tyrosine kinase: an amino terminal signal sequence followed by a long extracellular domain, a single hydrophobic transmembrane region and a cytoplasmic portion that contains a tyrosine kinase domain (Fig. 2B).

25 A survey for homologous proteins revealed that the predicted protein is most closely related to the two recently identified tyrosine kinases tie and tek (Partanen et al., 1992 Mol. Cell. Biol. 12:1698-1707; Dumont et al., 1992 Oncogene 7:1471-1480). With the
30 exception of two residues, the protein sequence published for tek is identical to the intracellular part of our polypeptide, from position 823 to the carboxyl-terminal residue 1123. Both an extracellular domain and a transmembrane region are missing in the
35 tek polypeptide (Fig. 3). It is therefore possible

that *tek* represents a partial sequence or that the mRNA encoding *tek* is the result of an alternative splice event, which results in the production of a cytoplasmic tyrosine kinase. Comparison of our protein with *tie* reveals a high degree of similarity especially in the cytoplasmic part (76% sequence
5 identify). In the extracellular domain and the transmembrane region, the similarity is less pronounced (33% and 37% respectively; Fig. 3). Ziegler et al. (1993 *Oncogene* 8:663-670) have also reported the cloning of a cDNA whose translation
10 product is a receptor tyrosine kinase with an overall similarity of more than 90% when compared to *tie-2* (Fig. 3). It is therefore likely that this protein, which they called *TEK*, is the human homolog of *tie-2*. The intracellular part of *tie-2* is also related to the
15 product of the human *ret* protooncogene and to FGF receptors (Takahashi et al., 1989 *Oncogene* 4:805-806; Partanen et al., 1991 *EMBO J.* 10:1347-1354; Stark et al., 1991 *Development* 113:641-651). The similarity to both is about 43%. The intracellular part can be
20 divided into three characteristic regions: the juxtamembrane sequence, the catalytic domain and the cytoplasmic tail. The kinase domain, which is split by an 14 amino acid insertion contains the GxGxxG consensus sequence that is part of the ATP binding
25 site. Typical tyrosine kinase motifs like HRDLAARN and DFGL are present.

Within the extracellular part, the FASTA program detected homologies to proteins including TAN-1
30 (Ellisen et al., 1991 *Cell* 66:649-661), Xotch (Coffman et al., 1990 *Science* 249:1438-1440), Laminin (Sasaki et al., 1988 *J. Biol. Chem.* 263:16536-16544). Delta (Vässin et al., 1987 *EMBO J.* 6:3431-3440) and Perlecan (Noonan et al., 1991 *J. Biol. Chem.* 266:22939-22947)
35 (around 30% sequence identity). The structural basis for these homologies are three EGF-like repeats in the

center of the extracellular portion of *tie-2* (Fig. 2A and 2B). EGF-like repeats consist of 30 to 40 amino acid residues often found in the extracellular parts of membrane-bound proteins or secreted proteins (Davies, 1990 New Biol. 5:410-419). A common feature of these domains are six conserved cysteine residues, which are known to be involved in disulfide bonds. The three EGF domains in *tie-2* contain two additional cysteine residues at the carboxyl-terminal end of each repeat.

The central EGF-like repeats are flanked by two different structural motifs, a single amino-terminal immunoglobulin (Ig) domain and a carboxyl-terminal triplet of fibronectin (FN) type III domains. Although the Ig domain does not exhibit clear sequence similarities to other known proteins (except for *tie* and *TEK*), the Ig domain has the conserved features of a C2-set domain including typical residues surrounding the first cysteine residue and the canonical GXYXC found at the second cysteine residue (Williams and Barclay, 1988 Ann. Rev. Immunol. 6:381-405).

The three fibronectin type III (FN III) domains are most closely related to FN III repeats present in the protein-tyrosine phosphatases Delta (Krueger et al., 1990 EMBO J. 9:3241-3252), LAR (Streuli et al., 1988 J. Exp. Med. 168:1553-1562) and DLAR (Streuli et al., 1989) and to FN III repeats of the axonal glycoprotein TAG-1 (Furley et al., 1990 Cell 61:157-170) (around 20% sequence identity). These domains have been described as units of approximately 90 amino acids containing hydrophobic residues at characteristic positions. In addition, a total of nine potential N-glycosylation sites are located in the extracellular part of *tie-2*.

6.2.2. TIE-2 EXPRESSION IN BRAIN CAPILLARIES

The amount of *tie-2* in mRNA from P4-P8 brain capillaries was compared with total P4 brain by Northern analysis. To avoid artifactual results, the part of the cDNA that encodes the least conserved regions of the protein, namely the three FN III domains, the transmembrane region and the juxtamembrane portion was used. Fig. 4 demonstrates the presence of a 4.7 kd mRNA highly enriched in the capillary fraction. RNA expression was also detected in organs like brain, kidney and heart.

6.2.3. TIE-2 EXPRESSION DURING BRAIN DEVELOPMENT

Investigation of the spatial and temporal expression profile of *tie-2* during brain development was performed by in situ hybridization. When analyzed, it became evident that *tie-2* mRNA is exclusively synthesized in the vasculature of (Fig. 5A-H) adult brain, P4 brain, E12.5 brain and E8.5 neuroectoderm. No other brain components were labelled. In P4 brain, *tie-2* expression was detected in capillaries that are about to invade the neural tissue as well as in vessels that have already reached deeper layers of the neuroectoderm. High magnifications in Fig. 5G,H clearly show the high concentration of silver grains over the vascular perikarya. In addition to capillaries, meningeal blood vessels and the choroid plexi were also found to synthesize *tie-2* mRNA at comparable levels (Figs 5, 7B). At E12.5 the overall expression pattern was virtually identical to that observed at P4, although the density of labelled structures in E12.5 brain was reduced (Fig. 5C,D). This observation correlates with the less extensive vascularization of the embryonic brain (Bär, 1980 Adv. Anat. Embryol. Cell Biol 59:1-

62). No hybridization signals were observed in the neuroectoderm of E8.5 embryos, because at that stage this tissue is still avascular (Fig. 8). In the adult brain, *tie-2* expression was detectable, although it seemed to be reduced when compared to postnatal or embryonic stages. Hybridization signals persisted over larger vessels especially those of the meninges (Fig. 5A,B). The synthesis of *tie-2* mRNA in smaller vessels and capillaries was barely detectable.

6.2.4. ENDOTHELIAL CELL-SPECIFIC EXPRESSION OF TIE-2

To identify the cellular source of *tie-2* mRNA, *in situ* hybridization and immunohistochemistry on adjacent sections were performed. The pattern of *tie-2* hybridization signals was compared with the immunohistochemical staining of a monoclonal antibody against the endothelial cell-specific adhesion molecule PECAM (CD31) (Newman et al., 1990, Science 247:1219-1222). Fig. 6 shows a representative example of one such experiment. The antibody stains the continuous layer of endothelial cells surrounding the lumen of a medium-sized blood vessel in the head region of an E12.5 embryo. On the adjacent section, the *tie-2*-specific probe labels the vessel in an identical way. These results, together with the northern hybridization signal detected in RNA from a capillary fraction highly enriched for endothelial cells, provide strong evidence for endothelium-specific expression of *tie-2* and PECAM in several organs and tissues. It is clear that *tie-2* mRNA is present in endothelial cells all over the body. Strong hybridization signals were associated with the heart endocardium as well with the myocardial blood vessels (Fig. 7E,F). The same holds true for the endothelium of the dorsal aorta (Fig. 7C,D), the intersomitic vasculature, the vessels surrounding the

lung bronchia and the capillaries perforating the spinal cord (Fig. 7H,D). In summary, *tie-2* gene expression seems to be a general feature of endothelial cells.

5 6.2.5. *TIE-2* EXPRESSION DURING
 EARLY STAGES OF DEVELOPMENT

10 The possible role of *tie-2* in early stages of vascular development was investigated by *in situ* hybridization studies with E8.5 sections. The probe detected *tie-2* mRNA in the mesodermal component of the
15 yolk sac (Fig. 8). This finding is of particular interest, because it is in the mesodermal component of the yolk sac that the first signs of blood vessel development are evident. Clusters of mesenchymal
20 cells form the so-called blood islands. At the margin of these aggregates cells, the so-called angioblasts, adopt an endothelial-like phenotype, whereas in the center cells differentiate into embryonic hemoblasts. Fig. 8 demonstrates that the peripheral angioblasts
25 synthesize high levels of *tie-2* mRNA.

30 Within the embryo proper, expression was seen in the anlagen of the vascular system, e.g. in the developing endocardium, the dorsal aortae and the cardinal veins (Fig. 8A,B). It is likely that these
35 signals stem from intraembryonic endothelial cell precursors, which are present in these structures at that stage, *tie-2* expression could also be detected in the allantois and in blood vessels of the maternal decidua.

30

35

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Risau, Werner
Schnurch, Harald
- (ii) TITLE OF INVENTION: TIE-2 A NOVEL TYROSINE RECEPTOR KINASE
- (iii) NUMBER OF SEQUENCES: 5
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Pennie & Edmonds
(B) STREET: 1155 Avenue of the Americas
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 10036-2711
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/152,552
(B) FILING DATE: 12-NOV-1993
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Coruzzi, Laura A.
(B) REGISTRATION NUMBER: 30,742
(C) REFERENCE/DOCKET NUMBER: 7683-059
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 790-9090
(B) TELEFAX: (212) 869-9741
(C) TELEX: 66141 PENNIE

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4640 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 341..3712

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TGCTCATCTG	TGGACGCTGG	ATGGGGAGAT	CTGGGGAAGT	ATGGACTCTT	TAGCCGGCTT	360
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 CTTGTAGGTG AATAAATGTC 4640

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1123 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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 Pro Leu Val Ser Asp Ala Glu Thr Ser Leu Thr Cys Ile Ala Ser Gly
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 50 55 60
 Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
 65 70 75 80
 Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile
 85 90 95
 Asn Gly Ala Tyr Phe Cys Glu Gly Arg Val Arg Gly Gln Ala Ile Arg
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 115 120 125
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Leu	Val	Lys	Pro	Asp	Gly	Thr	Val	Leu	Gln	Pro	Asn	Asp	Phe	Asn	Tyr
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 900 905 910
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His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Gly Glu Asn Tyr Ile 965 970 975		
Ala Lys Ile Ala Asp Phe Gly Leu Ser Arg Gly Gln Glu Val Tyr Val 980 985 990		
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Leu Asn Tyr Ser Val Tyr Thr Thr Asn Ser Asp Val Trp Ser Tyr Gly 1010 1015 1020		
Val Leu Leu Trp Glu Ile Val Ser Leu Gly Gly Thr Pro Tyr Cys Gly 1025 1030 1035 1040		
Met Thr Cys Ala Glu Leu Tyr Glu Lys Leu Pro Gln Gly Tyr Arg Leu 1045 1050 1055		
Glu Lys Pro Leu Asn Cys Asp Asp Glu Val Tyr Asp Leu Met Arg Gln 1060 1065 1070		
Cys Trp Arg Glu Lys Pro Tyr Glu Arg Pro Ser Phe Ala Gln Ile Leu 1075 1080 1085		
Val Ser Leu Asn Arg Met Leu Glu Glu Arg Lys Thr Tyr Val Asn Thr 1090 1095 1100		
Thr Leu Tyr Glu Lys Phe Thr Tyr Ala Gly Ile Asp Cys Ser Ala Glu 1105 1110 1115 1120		
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 301 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ile Lys Phe Gln Asp Val Ile Gly Glu Gly Asn Phe Gly Gln Val Leu 1 5 10 15
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Arg Met Lys Glu Tyr Ala Ser Lys Asp Asp His Arg Asp Phe Ala Gly 35 40 45
Glu Leu Glu Val Leu Cys Lys Leu Gly His His Pro Asn Ile Ile Asn

50	55	60
Leu 65	Leu Gly Ala Cys Glu 70	His Arg Gly Tyr Leu Tyr Leu Ala Ile Glu 80
Tyr 85	Ala Pro Met Gly Asn Leu Leu Asp 90	Phe Leu Arg Lys Ser Arg Val 95
Leu 100	Glu Thr Asp Pro Ala Phe Ala Ile 105	Ala Asn Ser Thr Ala Ser Thr 110
Leu 115	Ser Ser Gln Gln Leu Leu His Phe Ala Ala Asp 120	Val Ala Arg Gly 125
Met 130	Asp Tyr Leu Ser Gln Lys Gln Phe Ile His Arg Asp 140	Leu Ala Ala 145
Arg 145	Asn Ile Leu Val Gly Glu Asn Tyr Ile Ala Lys Ile Ala Asp Phe 150	155 160
Gly 165	Leu Ser Arg Gly Gln Glu Val Tyr Val Lys Lys Ile Met Gly Arg 170	175
Leu 180	Pro Val Arg Trp Met Ala Ile Glu Ser Leu Asn Tyr Ser Val Tyr 185	190
Thr 195	Thr Asn Ser Asp Val Trp Ser Tyr Gly Val Leu Leu Trp Glu Ile 200	205
Val 210	Ser Leu Gly Gly Thr Pro Tyr Cys Gly Met Thr Cys Ala Glu Leu 215	220
Tyr 225	Glu Lys Leu Pro Gln Gly Tyr Arg Leu Glu Lys Pro Leu Asn Cys 230	235 240
Asp 245	Asp Glu Val Tyr Asp Leu Met Arg Gln Cys Trp Arg Glu Lys Pro 250	255
Tyr 260	Glu Arg Pro Ser Phe Ala Gln Ile Leu Val Ser Leu Asn Arg Met 265	270
Leu 275	Glu Glu Arg Lys Thr Tyr Val Asn Thr Thr Leu Tyr Glu Lys Phe 280	285
Thr 290	Tyr Ala Gly Ile Asp Cys Ser Ala Glu Glu Ala Ala 295	300

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1124 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met 1	Asp 5	Ser 10	Leu 15	Ala 20	Ser 25	Leu 30	Val 35	Leu 40	Cys 45	Gly 50	Val 55	Ser 60	Leu 65	Leu 70
Ser Gly Thr Val Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu														

20					25					30					
Pro	Leu	Val	Ser	Asp	Ala	Glu	Thr	Ser	Leu	Thr	Cys	Ile	Ala	Ser	Gly
		35					40					45			
Trp	Arg	Pro	His	Glu	Pro	Ile	Thr	Ile	Gly	Arg	Asp	Phe	Glu	Ala	Leu
	50					55					60				
Met	Asn	Gln	His	Gln	Asp	Pro	Leu	Glu	Val	Thr	Gln	Asp	Val	Thr	Arg
65					70					75					80
Glu	Trp	Ala	Lys	Lys	Val	Val	Trp	Lys	Arg	Glu	Lys	Ala	Ser	Lys	Ile
			85						90					95	
Asn	Gly	Ala	Tyr	Phe	Cys	Glu	Gly	Arg	Val	Arg	Gly	Glu	Ala	Ile	Arg
			100					105					110		
Ile	Arg	Thr	Met	Lys	Met	Arg	Gln	Gln	Ala	Ser	Phe	Leu	Pro	Ala	Thr
		115					120					125			
Leu	Thr	Met	Thr	Val	Asp	Lys	Gly	Asp	Asn	Val	Asn	Ile	Ser	Phe	Lys
	130					135					140				
Lys	Val	Leu	Ile	Lys	Glu	Glu	Asp	Ala	Val	Ile	Tyr	Lys	Asn	Gly	Ser
145					150					155					160
Phe	Ile	His	Ser	Val	Pro	Arg	His	Glu	Val	Pro	Asp	Ile	Leu	Glu	Val
			165						170					175	
His	Leu	Pro	His	Ala	Gln	Pro	Gln	Asp	Ala	Gly	Val	Tyr	Ser	Ala	Arg
		180						185					190		
Tyr	Ile	Gly	Gly	Asn	Leu	Phe	Thr	Ser	Ala	Phe	Thr	Arg	Leu	Ile	Val
	195						200					205			
Arg	Arg	Cys	Glu	Ala	Gln	Lys	Trp	Gly	Pro	Glu	Cys	Asn	His	Leu	Cys
	210					215					220				
Thr	Ala	Cys	Met	Asn	Asn	Gly	Val	Cys	His	Glu	Asp	Thr	Gly	Glu	Cys
225				230						235					240
Ile	Cys	Pro	Pro	Gly	Phe	Met	Gly	Arg	Thr	Cys	Glu	Lys	Ala	Cys	Glu
				245					250					255	
Leu	His	Thr	Phe	Gly	Arg	Thr	Cys	Lys	Glu	Arg	Cys	Ser	Gly	Gln	Glu
		260						265					270		
Gly	Cys	Lys	Ser	Tyr	Val	Phe	Cys	Leu	Pro	Asp	Pro	Tyr	Gly	Cys	Ser
		275					280					285			
Cys	Ala	Thr	Gly	Trp	Lys	Gly	Leu	Gln	Cys	Asn	Glu	Ala	Cys	His	Pro
	290					295					300				
Gly	Phe	Tyr	Gly	Pro	Asp	Cys	Lys	Leu	Arg	Cys	Ser	Cys	Asn	Asn	Gly
305					310					315					320
Glu	Met	Cys	Asp	Arg	Phe	Gln	Gly	Cys	Leu	Cys	Ser	Pro	Gly	Trp	Gln
			325						330					335	
Gly	Leu	Gln	Cys	Glu	Arg	Glu	Gly	Ile	Pro	Arg	Met	Thr	Pro	Lys	Ile
		340						345					350		
Val	Asp	Leu	Pro	Asp	His	Ile	Glu	Val	Asn	Ser	Gly	Lys	Phe	Asn	Pro
		355					360					365			
Ile	Cys	Lys	Ala	Ser	Gly	Trp	Pro	Leu	Pro	Thr	Asn	Glu	Glu	Met	Thr
	370					375					380				

Leu Val Lys Pro Asp Gly Thr Val Leu His Pro Lys Asp Phe Asn His
 385 390 395 400
 Thr Asp His Phe Ser Val Ala Ile Phe Thr Ile His Arg Ile Leu Pro
 405 410 415
 Pro Asp Ser Gly Val Trp Val Cys Ser Val Asn Thr Val Ala Gly Met
 420 425 430
 Val Glu Lys Pro Phe Asn Ile Ser Val Lys Val Leu Pro Lys Pro Leu
 435 440 445
 Asn Ala Pro Asn Val Ile Asp Thr Gly His Asn Phe Ala Val Ile Asn
 450 455 460
 Ile Ser Ser Glu Pro Tyr Phe Gly Asp Gly Pro Ile Lys Ser Lys Lys
 465 470 475 480
 Leu Leu Tyr Lys Pro Val Asn His Tyr Glu Ala Trp Gln His Ile Gln
 485 490 495
 Val Thr Asn Glu Ile Val Thr Leu Asn Tyr Leu Glu Pro Arg Thr Glu
 500 505 510
 Tyr Glu Leu Cys Val Gln Leu Val Arg Arg Gly Glu Gly Gly Glu Gly
 515 520 525
 His Pro Gly Pro Val Arg Arg Phe Thr Thr Ala Ser Ile Gly Leu Pro
 530 535 540
 Pro Pro Arg Gly Leu Asn Leu Leu Pro Lys Ser Gln Thr Thr Leu Asn
 545 550 555 560
 Leu Thr Trp Gln Pro Ile Phe Pro Ser Ser Glu Asp Asp Phe Tyr Val
 565 570 575
 Glu Val Glu Arg Arg Ser Val Gln Lys Ser Asp Gln Gln Asn Ile Lys
 580 585 590
 Val Pro Gly Asn Leu Thr Ser Val Leu Leu Asn Asn Leu His Pro Arg
 595 600 605
 Glu Gln Tyr Val Val Arg Ala Arg Val Asn Thr Lys Ala Gln Gly Glu
 610 615 620
 Trp Ser Glu Asp Leu Thr Ala Trp Thr Leu Ser Asp Ile Leu Pro Pro
 625 630 635 640
 Gln Pro Glu Asn Ile Lys Ile Ser Asn Ile Thr His Ser Ser Ala Val
 645 650 655
 Ile Ser Trp Thr Ile Leu Asp Gly Tyr Ser Ile Ser Ser Ile Thr Ile
 660 665 670
 Arg Tyr Lys Val Gln Gly Lys Asn Glu Asp Gln His Val Asp Val Lys
 675 680 685
 Ile Lys Asn Ala Thr Ile Ile Gln Tyr Gln Leu Lys Gly Leu Glu Pro
 690 695 700
 Glu Thr Ala Tyr Gln Val Asp Ile Phe Ala Glu Asn Asn Ile Gly Ser
 705 710 715 720
 Ser Asn Pro Ala Phe Ser His Glu Leu Val Thr Leu Pro Glu Ser Gln
 725 730 735
 Ala Pro Ala Asp Leu Gly Gly Gly Lys Met Leu Leu Ile Ala Ile Leu

740					745					750					
Gly	Ser	Ala	Gly	Met	Thr	Cys	Leu	Thr	Val	Leu	Leu	Ala	Phe	Leu	Ile
		755					760					765			
Ile	Leu	Gln	Leu	Lys	Arg	Ala	Asn	Val	Gln	Arg	Arg	Met	Ala	Gln	Ala
	770					775					780				
Phe	Gln	Asn	Val	Arg	Glu	Glu	Pro	Ala	Val	Gln	Phe	Asn	Ser	Gly	Thr
	785				790					795					800
Leu	Ala	Leu	Asn	Arg	Lys	Val	Lys	Asn	Asn	Pro	Asp	Pro	Thr	Ile	Tyr
				805					810					815	
Pro	Val	Leu	Asp	Trp	Asn	Asp	Ile	Lys	Phe	Gln	Asp	Val	Ile	Gly	Glu
			820					825					830		
Gly	Asn	Phe	Gly	Gln	Val	Leu	Lys	Ala	Arg	Ile	Lys	Lys	Asp	Gly	Leu
		835					840					845			
Arg	Met	Asp	Ala	Ala	Ile	Lys	Arg	Met	Lys	Glu	Tyr	Ala	Ser	Lys	Asp
	850					855					860				
Asp	His	Arg	Asp	Phe	Ala	Gly	Glu	Leu	Glu	Val	Leu	Cys	Lys	Leu	Gly
	865				870					875					880
His	His	Pro	Asn	Ile	Ile	Asn	Leu	Leu	Gly	Ala	Cys	Glu	His	Arg	Gly
				885					890					895	
Tyr	Leu	Tyr	Leu	Ala	Ile	Glu	Tyr	Ala	Pro	His	Gly	Asn	Leu	Leu	Asp
			900					905					910		
Phe	Leu	Arg	Lys	Ser	Arg	Val	Leu	Glu	Thr	Asp	Pro	Ala	Phe	Ala	Ile
		915					920					925			
Ala	Asn	Ser	Thr	Ala	Ser	Thr	Leu	Ser	Ser	Gln	Gln	Leu	Leu	His	Phe
						935					940				
Ala	Ala	Asp	Val	Ala	Arg	Gly	Met	Asp	Tyr	Leu	Ser	Gln	Lys	Gln	Phe
	945				950					955					960
Ile	His	Arg	Asp	Leu	Ala	Ala	Arg	Asn	Ile	Leu	Val	Gly	Glu	Asn	Tyr
				965					970					975	
Val	Ala	Lys	Ile	Ala	Asp	Phe	Gly	Leu	Ser	Arg	Gly	Gln	Glu	Val	Tyr
			980					985					990		
Val	Lys	Lys	Thr	Met	Gly	Arg	Leu	Pro	Val	Arg	Trp	Met	Ala	Ile	Glu
		995					1000					1005			
Ser	Leu	Asn	Tyr	Ser	Val	Tyr	Thr	Thr	Asn	Ser	Asp	Val	Trp	Ser	Tyr
	1010					1015					1020				
Gly	Val	Leu	Leu	Trp	Glu	Ile	Val	Ser	Leu	Gly	Gly	Thr	Pro	Tyr	Cys
	1025				1030					1035					1040
Gly	Met	Thr	Cys	Ala	Glu	Leu	Tyr	Glu	Lys	Leu	Pro	Gln	Gly	Tyr	Arg
				1045					1050					1055	
Leu	Glu	Lys	Pro	Leu	Asn	Cys	Asp	Asp	Glu	Val	Tyr	Asp	Leu	Met	Arg
			1060					1065					1070		
Gln	Cys	Trp													

Thr Thr Leu Tyr Glu Lys Phe Thr Tyr Ala Gly Ile Asp Cys Ser Ala
 1105 1110 1115 1120
 Glu Glu Ala Ala

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1138 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Val Trp Arg Val Pro Pro Phe Leu Leu Pro Ile Leu Phe Leu Ala
 1 5 10 15
 Ser His Val Gly Ala Ala Val Asp Leu Thr Leu Leu Ala Asn Leu Arg
 20 25 30
 Leu Thr Asp Pro Gln Arg Phe Phe Leu Thr Cys Val Ser Gly Glu Ala
 35 40 45
 Gly Ala Gly Arg Gly Ser Asp Ala Trp Gly Pro Pro Leu Leu Leu Glu
 50 55 60
 Lys Asp Asp Arg Ile Val Arg Thr Pro Pro Gly Pro Pro Leu Arg Leu
 65 70 75 80
 Ala Arg Asn Gly Ser His Gln Val Thr Leu Arg Gly Phe Ser Lys Pro
 85 90 95
 Ser Asp Leu Val Gly Val Phe Ser Cys Val Gly Gly Ala Gly Ala Arg
 100 105 110
 Arg Thr Arg Val Ile Tyr Val His Asn Ser Pro Gly Ala His Leu Leu
 115 120 125
 Pro Asp Lys Val Thr His Thr Val Asn Lys Gly Asp Thr Ala Val Leu
 130 135 140
 Ser Ala Arg Val His Lys Glu Lys Gln Thr Asp Val Ile Trp Lys Ser
 145 150 155 160
 Asn Gly Ser Tyr Phe Tyr Thr Leu Asp Trp His Glu Ala Gln Asp Gly
 165 170 175
 Arg Phe Leu Leu Gln Leu Pro Asn Val Gln Pro Pro Ser Ser Gly Ile
 180 185 190
 Tyr Ser Ala Thr Tyr Leu Glu Ala Ser Pro Leu Gly Ser Ala Phe Phe
 195 200 205
 Arg Leu Ile Val Arg Gly Cys Gly Ala Gly Arg Trp Gly Pro Gly Cys
 210 215 220
 Thr Lys Glu Cys Pro Gly Cys Leu His Gly Gly Val Cys His Asp His
 225 230 235 240

Asp Gly Glu Cys Val Cys Pro Pro Gly Phe Thr Gly Thr Arg Cys Glu
 245 250 255
 Gln Ala Cys Arg Glu Gly Arg Phe Gly Gln Ser Cys Gln Glu Gln Cys
 260 265 270
 Pro Gly Ile Ser Gly Cys Arg Gly Leu Thr Phe Cys Leu Pro Asp Pro
 275 280 285
 Tyr Gly Cys Ser Cys Gly Ser Gly Trp Arg Gly Ser Gln Cys Gln Glu
 290 295 300
 Ala Cys Ala Pro Gly His Phe Gly Ala Asp Cys Arg Leu Gln Cys Gln
 305 310 315 320
 Cys Gln Asn Gly Gly Thr Cys Asp Arg Phe Ser Gly Cys Val Cys Pro
 325 330 335
 Ser Gly Trp His Gly Val His Cys Glu Lys Ser Asp Arg Ile Pro Gln
 340 345 350
 Ile Leu Asn Met Ala Ser Glu Leu Glu Phe Asn Leu Glu Thr Met Pro
 355 360 365
 Arg Ile Asn Cys Ala Ala Ala Gly Asn Pro Phe Pro Val Arg Gly Ser
 370 375 380
 Ile Glu Leu Arg Lys Pro Asp Gly Thr Val Leu Leu Ser Thr Lys Ala
 385 390 395 400
 Ile Val Glu Pro Glu Lys Thr Thr Ala Glu Phe Glu Val Pro Arg Leu
 405 410 415
 Val Leu Ala Asp Ser Gly Phe Trp Glu Cys Arg Val Ser Thr Ser Gly
 420 425 430
 Gly Gln Asp Ser Arg Arg Phe Lys Val Asn Val Lys Val Pro Pro Val
 435 440 445
 Pro Leu Ala Ala Pro Arg Leu Leu Thr Lys Gln Ser Arg Gln Leu Val
 450 455 460
 Val Ser Pro Leu Val Ser Phe Ser Gly Asp Gly Pro Ile Ser Thr Val
 465 470 475 480
 Arg Leu His Tyr Arg Pro Gln Asp Ser Thr Met Asp Trp Ser Thr Ile
 485 490 495
 Val Val Asp Pro Ser Glu Asn Val Thr Leu Met Asn Leu Arg Pro Lys
 500 505 510
 Thr Gly Tyr Ser Val Arg Val Gln Leu Ser Arg Pro Gly Glu Gly Gly
 515 520 525
 Glu Gly Ala Trp Gly Pro Pro Thr Leu Met Thr Thr Asp Cys Pro Glu
 530 535 540
 Pro Leu Leu Gln Pro Trp Leu Glu Gly Trp His Val Glu Gly Thr Asp
 545 550 555 560
 Arg Leu Arg Val Ser Trp Ser Leu Pro Leu Val Pro Gly Pro Leu Val
 565 570 575
 Gly Asp Gly Phe Leu Leu Arg Leu Trp Asp Gly Thr Arg Gly Gln Glu
 580 585 590
 Arg Arg Glu Asn Val Ser Ser Pro Gln Ala Arg Thr Ala Leu Leu Thr

595					600					605					
Gly	Leu	Thr	Pro	Gly	Thr	His	Tyr	Gln	Leu	Asp	Val	Gln	Leu	Tyr	His
610						615					620				
Cys	Thr	Leu	Leu	Gly	Pro	Ala	Ser	Pro	Pro	Ala	His	Val	Leu	Leu	Pro
625					630					635					640
Pro	Ser	Gly	Pro	Pro	Ala	Pro	Arg	His	Leu	His	Ala	Gln	Ala	Leu	Ser
				645					650					655	
Asp	Ser	Glu	Ile	Gln	Leu	Thr	Trp	Lys	His	Pro	Glu	Ala	Leu	Pro	Gly
			660					665					670		
Pro	Ile	Ser	Lys	Tyr	Val	Val	Glu	Val	Gln	Val	Ala	Gly	Gly	Ala	Gly
			675				680					685			
Asp	Pro	Leu	Trp	Ile	Asp	Val	Asp	Arg	Pro	Glu	Glu	Thr	Ser	Thr	Ile
	690					695					700				
Ile	Arg	Gly	Leu	Asn	Ala	Ser	Thr	Arg	Tyr	Leu	Phe	Arg	Met	Arg	Ala
705					710					715					720
Ser	Ile	Gln	Gly	Leu	Gly	Asp	Trp	Ser	Asn	Thr	Val	Glu	Glu	Ser	Thr
			725						730					735	
Leu	Gly	Asn	Gly	Leu	Gln	Ala	Glu	Gly	Pro	Val	Gln	Glu	Ser	Arg	Ala
			740				745						750		
Ala	Glu	Glu	Gly	Leu	Asp	Gln	Gln	Leu	Ile	Leu	Ala	Val	Val	Gly	Ser
			755			760						765			
Val	Ser	Ala	Thr	Cys	Leu	Thr	Ile	Leu	Ala	Ala	Leu	Leu	Thr	Leu	Val
	770					775					780				
Cys	Ile	Arg	Arg	Ser	Cys	Leu	His	Arg	Arg	Arg	Thr	Phe	Thr	Tyr	Gln
785					790					795					800
Ser	Gly	Ser	Gly	Glu	Glu	Thr	Ile	Leu	Gln	Phe	Ser	Ser	Gly	Thr	Leu
				805					810					815	
Thr	Leu	Thr	Arg	Arg	Pro	Lys	Leu	Gln	Pro	Glu	Pro	Leu	Ser	Tyr	Pro
			820					825					830		
Val	Leu	Glu	Trp	Glu	Asp	Ile	Thr	Phe	Glu	Asp	Leu	Ile	Gly	Glu	Gly
			835				840					845			
Asn	Phe	Gly	Gln	Val	Ile	Arg	Ala	Met	Ile	Lys	Lys	Asp	Gly	Leu	Lys
	850					855					860				
Met	Asn	Ala	Ala	Ile	Lys	Met	Leu	Lys	Glu	Tyr	Ala	Ser	Glu	Asn	Asp
865						870				875				880	
His	Arg	Asp	Phe	Ala	Gly	Glu	Leu	Glu	Val	Leu	Cys	Lys	Leu	Gly	His
			885						890					895	
His	Pro	Asn	Ile	Ile	Asn	Leu	Leu	Gly	Ala	Cys	Lys	Asn	Arg	Gly	Tyr
			900					905					910		
Leu	Tyr	Ile	Ala	Ile	Glu	Tyr	Ala	Pro	Tyr	Gly	Asn	Leu	Leu	Asp	Phe
			915				920					925			
Leu	Arg	Lys	Ser	Arg	Val	Leu	Glu	Thr	Asp	Pro	Ala	Phe	Ala	Arg	Glu
	930					935					940				
His	Gly	Thr	Ala	Ser	Thr	Leu	Ser	Ser	Arg	Gln	Leu	Leu	Arg	Phe	Ala
945					950					955				960	

Ser Asp Ala Ala Asn Gly Met Gln Tyr Leu Ser Glu Lys Gln Phe Ile
 965 970 975
 His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Gly Glu Asn Leu Ala
 980 985 990
 Ser Lys Ile Ala Asp Phe Gly Leu Ser Arg Gly Glu Glu Val Tyr Val
 995 1000 1005
 Lys Lys Thr Met Gly Arg Leu Pro Val Arg Trp Met Ala Ile Glu Ser
 1010 1015 1020
 Leu Asn Tyr Ser Val Tyr Thr Thr Lys Ser Asp Val Trp Ser Phe Gly
 1025 1030 1035 1040
 Val Leu Leu Trp Glu Ile Val Ser Leu Gly Gly Thr Pro Tyr Cys Gly
 1045 1050 1055
 Met Thr Cys Ala Glu Leu Tyr Glu Lys Leu Pro Gln Gly Tyr Arg Met
 1060 1065 1070
 Glu Gln Pro Arg Asn Cys Asp Asp Glu Val Tyr Glu Leu Met Arg Gln
 1075 1080 1085
 Cys Trp Arg Asp Arg Pro Tyr Glu Arg Pro Pro Phe Ala Gln Ile Ala
 1090 1095 1100
 Leu Gln Leu Gly Arg Met Leu Glu Ala Arg Lys Ala Tyr Val Asn Met
 1105 1110 1115 1120
 Ser Leu Phe Glu Asn Phe Thr Tyr Ala Gly Ile Asp Ala Thr Ala Glu
 1125 1130 1135
 Glu Ala

WHAT IS CLAIMED IS:

1. An isolated nucleotide sequence encoding a *tie-2* protein.
- 5 2. A cDNA nucleotide sequence encoding a *tie-2* protein.
- 10 3. A cDNA nucleotide sequence encoding a member of the MCK-10 family of proteins in which the nucleotide sequence encodes the amino acid sequence of FIG. 2 (SEQ. ID NO:), or which is capable of selectively hybridizing to the DNA sequence of FIG. 1 (SEQ. ID NO:).
- 15 4. A recombinant DNA vector containing a nucleotide sequence that encodes a *tie-2* protein.
- 20 5. A recombinant DNA vector containing a nucleotide sequence that encodes a *tie-2* fusion protein.
- 25 6. The recombinant DNA vector of Claim 4 in which the *tie-2* nucleotide sequence is operatively associated with a regulatory sequence that controls the *tie-2* gene expression in a host.
- 30 7. The recombinant DNA vector of Claim 5 in which the *tie-2* fusion protein nucleotide sequence is operatively associated with a regulatory sequence that controls the *tie-2* fusion protein gene expression in a host.
- 35 8. The DNA of Claim 2, 3, 4, 5, 6 or 7 in which the nucleotide sequence is capable of hybridizing under standard conditions, or which would be capable of hybridizing under standard conditions but for the

degeneracy of the genetic code to the DNA sequence of FIG. 1.

9. An engineered host cell that contains the recombinant DNA vector of Claims 4, 5, 6, or 7.

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10. An engineered cell line that contains the recombinant DNA expression vector of Claim 6 and expresses *tie-2*.

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11. An engineered cell line that contains the recombinant DNA expression vector of Claim 7 and expresses *tie-2* fusion protein.

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12. The engineered cell line of Claim 10 which expresses the *tie-2* on the surface of the cell.

13. The engineered cell line of Claim 11 that expresses the *tie-2* fusion protein on the surface of the cell.

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14. A method for producing recombinant *tie-2*, comprising:

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(a) culturing a host cell transformed with the recombinant DNA expression vector of Claim 9 and which expresses the *tie-2*; and

(b) recovering the *tie-2* gene product from the cell culture.

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15. A method for producing recombinant *tie-2* fusion protein, comprising:

(a) culturing a host cell transformed with the recombinant DNA expression vector of Claim 11 and which expresses the *tie-2* fusion protein; and

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(b) recovering the *tie-2* fusion protein from the cell culture.

16. An isolated recombinant *tie-2* receptor protein.

17. A fusion protein comprising *tie-2* linked to a heterologous protein or peptide sequence.

5 18. An oligonucleotide which encodes an antisense sequence complementary to the *tie-2* nucleotide sequence, and which inhibits translation of the *tie-2* gene in a cell.

10 19. The oligonucleotide of Claim 18 which is complementary to a nucleotide sequence encoding the amino terminal region of the *tie-2*.

15 20. A monoclonal antibody which immunospecifically binds to an epitope of the *tie-2*.

20 21. The monoclonal antibody of Claim 20 which competitively inhibits the binding of ligand to the *tie-2*.

22. The monoclonal antibody of Claim 20 which is linked to a cytotoxic agent.

25 23. The monoclonal antibody of Claim 20 which is linked to a radioisotope.

24. A method for screening and identifying antagonists of *tie-2*, comprising:

- 30 (a) contacting a cell line that expresses *tie-2* with a test compound; and
(b) determining whether the test compound inhibits the binding of *tie-2* ligand and the cellular effects of ligand binding on the cell line,
- 35

in which antagonists are identified as those compounds that inhibit both the binding and cellular effects of tie-2 ligand binding on the cell line.

25. A method for screening and identifying agonists of tie-2 ligand, comprising:

- 5 (a) contacting a cell line that expresses the tie-2 with a test compound in the presence and in the absence of ligand;
- 10 (b) determining whether, in the presence of ligand, the test compound inhibits the binding of ligand to the cell line and;
- 15 (c) determining whether, in the absence of the ligand, the test compound mimics the cellular effects of ligand on the cell line

in which agonists are identified as those test compounds that inhibit the binding but mimic the cellular effects of ligand on the cell line.

26. The method according to Claim 24 or 25 in which the cell line is a genetically engineered cell line.

27. The method according to Claim 24 or 25 in which the cell line endogenously expresses the tie-2.

28. A method for screening and identifying antagonists of tie-2 activity comprising:

- 30 (a) contacting tie-2 protein with a random peptide library such that tie-2 will recognize and bind to one or more peptide species within the library;
- (b) isolating the tie-2/peptide combination;
- 35 (c) determining the sequence of the peptide isolated in step c; and

- (d) determining whether the test compound inhibits the biological activity of *tie-2*.

in which agonists are identified as those peptides that inhibit the binding but mimic the cellular effects of *tie-2*.

5

29. A method for screening and identifying agonists of *tie-2* comprising:

- (a) contacting *tie-2* protein with a random peptide library such that *tie-2* will recognize and bind to one or more peptide species within the library;
- (b) isolating the *tie-2*/peptide combination;
- (c) determining the sequence of the peptide isolated in step c; and
- (d) determining whether, in the absence of the *tie-2* ligand, the peptide mimics the cellular effects of *tie-2*.

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30. The method according to Claim 28 or 29 in which the *tie-2* protein is genetically engineered.

31. A method of modulating the endogenous enzymatic activity of the tyrosine kinase *tie-2* receptor in a mammal comprising administering to the mammal an effective amount of a ligand to the *tie-2* receptor protein to modulate the enzymatic activity.

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32. The method of Claim 31 in which the enzymatic activity of the receptor protein is decreased.

33. A recombinant vector containing a nucleotide sequence that encodes a truncated *tie-2* which has

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dominant-negative activity which inhibits the biological activity tie-2.

34. The recombinant vector of claim 33 in which the vector is a retrovirus vector.

5 35. An engineered cell line that contains the recombinant DNA vector of Claim 34 and expresses truncated tie-2.

10 36. An engineered cell line that contains the recombinant vector of Claim 35 and produces infectious retrovirus particles expressing truncated tie-2.

15 37. An isolated recombinant truncated tie-2 receptor protein which has dominant-negative activity which inhibits the biological activity of tie-2.

20 38. A method of modulating the biological activity of tie-2 in a mammal comprising administering to the mammal an effective amount of truncated tie-2 receptor protein which inhibits the biological activity of tie-2 activation.

25

30

35

AGCAGGAGCC GGAGCAGGAG CAGAAGATAA GCCTTGGATG AAGGGCAAGA TGGATAGGCC TCGCTCTGCC 70
CCAAGCCCTG CTGATACCAA GTGCCTTTAA GATACAGCCT TTCCCATCCT AATCTGCAAA GGAAACAGGA 140
AAAAGGAAct TAACCCTCCC TGTGCTCAGA CAGAAATGAG ACTGTTACCG CCTGCTTCTG TGGTGTCTT 210
CCTTGCCGCC AACTTGTAAG CAAGAGCGAG TGGACCATGC GAGCGGGAAG TCGCAAAGTT GTGAGTTGTT 280
GAAAGCTTCC CAGGGACTCA TGCTCATCTG TGGACGCTGG ATGGGGAGAT CTGGGGAAGT ATGGACTCTT 350
TAGCCGGCTT AGTTCTCTGT GGAGTCAGCT TGCTCCTTTA TGGAGTAGTA GAAGGTGCCA TGGACCTGAT 420
CTTGATCAAT TCCCTACCTC TTGTGTCTGA TCCCGAAACA TCCCTCACCT GCATTGCCCTC TGGGTGGCAC 490
CCCCATGAGC CCATCACCAT AGGAAGGGAC TTTGAAGCCT TAATGAACCA GCACCAAGAT CCACTGGAGG 560
TACTCAAGA TGTGACCAGA GAATGGGCGA AAAAAGTTGT TTGAAGAGA GAAAAGGCCA GTAAGATTAA 630
TGGTGCTTAT TTCTGTGAAG GTCGAGTTCC AGGACAGGCT ATAAGGATAC GGACCATGAA GATGCGTCAA 700
CAAGCGTCCT TCCTACCTGC TACTTTAACT ATGACCGTGG ACAGGGGAGA TAATGTGAAC ATATCTTTCA 770
AAAAGGTGTT AATTAAAGAA GAAGATGCAG TGATTACAA AAATGGCTCC TTCATCCACT CAGTCCCCCG 840
GCATGAAGTA CCTGATATTT TAGAAGTTCA CTTGCCGCAT GCTCAGCCCC AGGATGCTGG TGTGTACTCG 910
GCCAGGTACA TAGGAGGAAA CCTGTTACCC TCAGCCTTCA CCAGGCTGAT TGTTCGGAGA TGTGAAGCTC 980
AGAAGTGGGG CCCCAGCTGT AGCCGTCCTT GTACTACTTG CAAGAACAAT GGAGTCTGCC ATGAAGATAC 1050
CGGGGAATGC ATTTGCCCTC CTGGGTTTAT GGGGAGAACA TGTGAGAAAG CTGTGAGCC GCACACATTT 1120
GGCAGGACCT GTAAAGAAAG GTGTAGTGA CCAGAAGGAT GCAAGTCTTA TGTGTTCTGT CTCCCAGACC 1190
CTTACGGGTG TTCCTGTGCC ACAGGCTGGA GGGGGTTGCA GTGCAATGAA GCATGCCCAT CTGGTTACTA 1260
CGGACCAGAC TGTAAGCTCA GGTGCCACTG TACCAATGAA GAGATATGTG ATCGGTTCCA AGGATGCCTC 1330
TGCTCTCAAG GATGGCAAGG GCTGCAGTGT GAGAAAGAAG GCAGGCCAAG GATGACTCCA CAGATAGAGG 1400
ATTGCCAGA TCACATTGAA GTAAACAGTG GAAAATTTAA CCCCATCTGC AAAGCCTCTG GGTGGCCACT 1470

FIG.1A

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SUBSTITUTE SHEET (RULE 26)

ACCTACTAGT GAAGAAATGA CCCTAGTGAA GCCAGATGGG ACAGTGCTCC AACCAAATGA CTTCAACTAT 1540
ACAGATCGTT TCTCAGTGGC CATATTCAGT GTCAACCGAG TCTTACCTCC TGAATCAGGA GTCTGGGTCT 1610
GCAGTGTGAA CACAGTGGCT GGGATGGTGG AAAAGCCTTT CAACATTTCC GTCAAAGTTC TTCCAGAGCC 1680
CCTGCACGCC CCAAATGTGA TTGACACTGG ACATAACTTT GCTATCATCA ATATCAGCTC TGAGCCTTAC 1750
TTTGGGGATG GACCCATCAA ATCCAAGAAG CTTTCTATA AACCTGTCAA TCAGGCCTGG AAATACATTG 1820
AAGTGACGAA TGAGATTTTC ACTCTCAACT ACTTGAGGCC GCGGACTGAC TACGAGCTGT GTGTGCAGCT 1890
GGCCCGTCCT GGAGAGGGTG GAGAAGGGCA TCCTGGGCCT GTGAGACGAT TTACAACAGC GTCTATCGGA 1960
CTCCCTCCTC CAAGAGGTCT CAGTCTCCTG CCAAAAAGCC AGACAGCTCT AAATTGACT TGGCAACCGA 2030
TATTTACAAA CTCAGAAGAT GAATTTTATG TGAAGTGA GAGGCGATCC CTGCAAACAA CAAGTGATCA 2100
GCAGAACATC AAAGTGCCTG GGAACCTGAC CTCGGTGCTA CTGAGCAACT TAGTCCCCAG GGAGCAGTAC 2170
ACAGTCCGAG CTAGAGTCAA CACCAAGGCG CAGGGGGAGT GGAGTGAAGA ACTCAGGGCC TGGACCCTTA 2240
GTGACATTCT CCTCCTCAA CCAGAAAACA TCAAGATCTC CAACATCACT GACTCCACAG CTATGGTTTC 2310
TTGGACAATA GTGGATGGCT ATTCGATTTC TTCCATCATC ATCCGTATA AGGTCAGGG CAAAATGAA 2380
GACCAGCACA TTGATGTGAA GATCAAGAAT GCTACCGTTA CTCAGTACCA GCTCAAGGGC CTAGAGCCAG 2450
AGACTACATA CCATGTGGAT ATTTTGTG AGAACAACAT AGGATCAAGC AACCAGCCT TTTCTCATGA 2520
ACTGAGGACG CTTCCACATT CCCCAGCCTC TGCAGACCTC GGAGGGGGAA AGATGCTACT CATAGCCATC 2590
CTTGGGTCCG CTGGAATGAC TTGCATCACC GTGCTGTTGG CGTTTCTGAT TATGTTGCAA CTGAAGAGAG 2660
CAAATGTCCA AAGGAGAATG GCTCAGGCAT TCCAGAACGT GAGAGAAGAA CCAGCTGTGC AGTTTAACTC 2730
AGGAACTCTG GCCCTTAACA GGAAGGCCAA AAACAATCCG GATCCACAA TTTATCCTGT GCTTGACTGG 2800
AATGACATCA AGTTTCAAGA CGTGATCGGA GAGGGCAACT TTGGCCAGGT TCTGAAGGCA CGCATCAAGA 2870
AGGATGGGTT ACGGATGGAT GCGGCCATCA AGAGGATGAA AGAGTATGCC TCCAAAGATG ATCACAGGGA 2940
CTTCGCAGGA GAACTGGAGG TTCTTTGTAA ACTTGACAC CATCCAAACA TCATCAATCT CTTGGGAGCA 3010

FIG.1B

TGTGAACACC GAGGCTATTT GTACCTAGCT ATTGAGTATG CCCCGCATGG AAACCTCCTG GACTTCCTGC 3080
GTAAGAGCAG AGTGCTAGAG ACAGACCCTG CTTTGGCCAT CGCCAACAGT ACAGCTTCCA CACTGTCCTC 3150
CCAACAGCTT CTTCAATTTG CTGCAGATGT GGGCCGGGGG ATGGACTACT TGAGCCAGAA ACAGTTTATC 3220
CACAGGGACC TGGCTGCCAG AAACATTTTA GTTGGTGAAA ACTACATAGC CAAAATAGCA GATTTTGGAT 3290
TGTCACGAGG TCAAGAAGTG TATGTGAAAA AGACAATGGG AAGGCTCCCA GTGCGTTGGA TGGCAATCGA 3360
ATCACTGAAC TATAGTGTCT ATACAACCAA CAGTGATGTC TGGTCCTATG GTGTATTGCT CTGGGAGATT 3430
GTTAGCTTAG GAGGCACCCC CTACTGCGGC ATGACGTGGC CGGAGCTCTA TGAGAAGCTA CCCCAGGGCT 3500
ACAGGCTGGA GAAGCCCCTG AACTGTGATG ATGAGGTGTA TGATCTAATG AGACAGTGCT GGAGGGAGAA 3570
GCCTTATGAG AGACCATCAT TTGCCAGAT ATTGGTGTCC TTAACAGGA TGCTGGAAGA ACGGAAGACA 3640
TACGTGAACA CCACACTGTA TGAGAAGTTT ACCTATGCAG GAATTGACTG CTCTGCGGAA GAAGCAGCCT 3710
AGAGCAGAAC TCTTCATGTA CAACGGCCAT TTCTCCTCAC TGGCGCGAGA GCGCCTTGAC ACCTGTACCA 3780
AGCAAGCCAC CCACTGCCAA GAGATGTGAT ATATAAGTG ATATATTGTG CTGTGTTTGG GACCCTCCTC 3850
ATACAGCTCG TCGGATCTG CAGTGTGTTT TGA CTCTAAT GTGACTGTAT ATACTGCTCG GAGTAAGAAT 3920
GTGCTAAGAT CAGAATGCCT GTTCGTGGTT TCATATAATA TATTTTCTA AAAGCATAGA TTGCACAGGA 3990
AGGTATGAGT ACAAATACTG TAATGCATAA CTTGTTACTG TCCTAGATGT GTTGATATT TTTCCTTTAC 4060
AACTGAATGC TATAAAAGTG TTTGCTGTG TACACATAAG ATACTGTTG TTAATAAAG CATTCCCTTG 4130
ACAGCACAGG AAGAAAAGCG AGGGAAATGT ATGGATTATA TAAATGTGG GTTACTACAC AAGAGGCCGA 4200
ACATTCCAAG TAGCAGAAGA GAGGGTCTCT CAACTCTGCT CCTCACCTGC AGAAGCCAGT TTGTTTGGCC 4270
ATGTGACAAT TGTCTGTGT TTTTATAGCA CCCAAATCAT TCTAAATAT GAACATCTAA AAACCTTGCT 4340
AGGAGACTAA GAACCTTTGG AGAGATAGAT ATAAGTACCG TCAAAAACA AAACGCGGG ACTTACATT 4410
ATTTTCTATA GTAATCTGTT GTACATTTA AGAAGTAAAA CTAGGAATTT AGGAGTGATG TGTGACATT 4480
CTGACATGGA GTTACCATCC CCACATGTAT CACATACTGT CATATTCCCA CATGTATCAC ACATGTATTG 4550
TAAATTTTG TAGTTTGTAT CACTGTGAA TTTACTGTTG ATGTGGTAGC CACCTGCTGC AATGGTTCCT 4620
CTTGTAGGTG AATAAATGTC

FIG.1C

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SUBSTITUTE SHEET (RULE 26)

MDSLGLVLC GVSLLLYGVV EGAMDLILIN SLPLVSDAET SLTCIASGWH PHEPITIGRD 60
 FEALMNHQD PLEVTDQVTR EWAKKVVWKR EKASKINGAY FCEGRVGRQA IRIRTMKMRQ 120
 QASFLPATLT MTVDRGDNVN ISFKKVLKE EDAVIYKNGS FIHSVPRHEV PDILEVHLPH 180
 AQPQDAGVYS ARYIGGNLFT SAFTRLIVRR ¹CEAQKWGPDC SRPCTTCKNN GVCHEDTGEC 240
²ICPPGFMGRT CEKACEPHTF GRICKERCSCG PEGCKSYVFC LPDPYGCSCA TGRGLQONE 300
³ACPSGYYGPD CKLRCHCTNE EICDRFQGCL CSQGWQGLQC EKEGRPRMTP QIEDLPDHE 360
 VNSGKFNPIK KASGWPLPTS EEMTLVKPDG TVLQPNDFNY TDRFSVAIFT VNRVLPPDSG 420
¹WVCSVNTVA GMVEKPFNIS VKVLEPLHA PNVIDTGHNF AIINISSEPY FGDGPIKSKK 480
²LFYKPVNQAW KYIEVTNEIF TLNLEPRTD YELCVQLARP GEGGEGHPGP VRRFTTASIG 540
 LPPRGLSLL PKSQTALNLT WQIFITNSED EFYVEVERRS LOTTSDQONI KVPGNLTSVL 600
³LSNLVPREQY TVRARVNTKA QGEWSEELRA WTLSDILPPQ PENIKISNIT DSTAMVSWTI 660
 VDGYSISSII IRYKVQCKNE DQHIDVKIKN ATVTQYQLKG LEPETTYHVD IFAENNIGSS 720
 NPAFSHELRT LPHSPASADL GGGKMLLIAI LGSAGMTCIT VLLAFLIMLQ LKRANVQRRM 780
 AQAFQNVREE PAVQFNSGTL ALNRKAKNNP DPTIYPVLDW NDIKFQDVIG EGNFGQVLKA 840
 RIKKDGLRMD AAIKRMKEYA SKDDHRDFAG ELEVLCKLGH HPNIINLLGA CEHRGYLYLA 900
 IEYAPHGNLL DFLRKSRLVE TDPAFAIANS TASTLSSQQL LHFAADVARG MDYLSQKQFI 960
 HRDLAARNIL VGENYIAKIA DFGLSRGQEV YVKKTMGRLP VRIMATESLN YSVYTTNSDV 1020
 WSYGVLLWEI VSLGGTPYCG MTCAELYEKL PQGYRLEKPL NCODEVYDLM ROCWREKPYE 1080
 RPSFAQILVS LNRMLEERKT YVNTTLYEKF TYAGIDCSAE EAA

FIG.2A

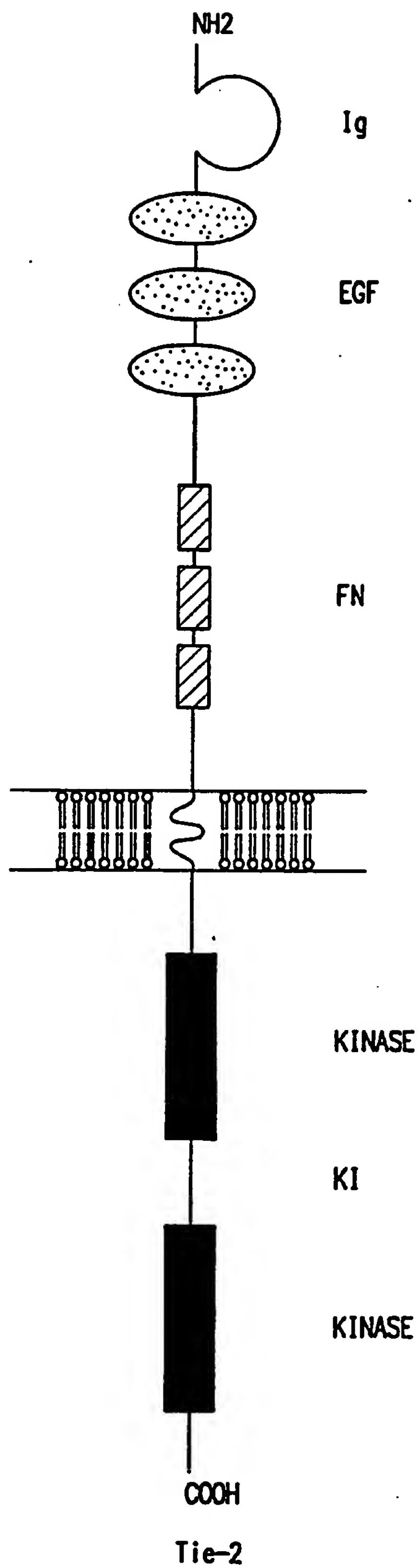


FIG.2B

1 50 100
lie-2 .MDSLAGLV CGVSLLYGV VEGAMDIL I NSLPLVSDAE TSLICIASGW HPHEPITIGR DFEALMNOHQ DPLEVTQDVT REWAKK.... VWIKREKAS
TEK .---S---S-T---R---
lie MWRVPPFL-..PI-f-ASH -CA-V-T-L AN-R-TDQR FF---V---E AGAGRGSDAW GPPL-LEKDD RIVRTPPGPP LRL-RNGSHQ VTLRGFS-P-

101 150 200
lie-2 KINGAYCEG RVRGQAIRIR TMKMRQQASF LPATLTMTVD RGDNNISFK KVLKEEDAV IYKNGSF IHS VPRHEVPD.I LEVHLPHAQP QDAGVYSARY
TEK ---E---K-VN---
lie DLV-VFS-V- GAGART-VI YHNSPG-HL -DKV-H-N K-TAVL-AR VHKE-QT-VI WKS---YFYT LDW-AQ-GR FLLO-NV- PSS-I-T-

201 250 300
lie-2 ICGNLTSAF TRLIVRCEA QKNGPDCSRP CITCKNNGVC HEDTGEICP PGFMGRTECK ACEPHTFGRT CKERCSGPEG CKSYVFLCPD PYGCSCATGW
TEK ---E-NHL-A-M---L---Q---
lie LEASPLG---F---G-G-GR---G-TKE -PG-LHG---DHD-V- ---T-IR-Q -REGR-QS -Q-Q-PCIS- RGLT---CS-

301 350 400
lie-2 RGLQCNEACP SGYCGPDCKL RCHCTNEEIC DRFGQCCLCSQ GWGGLQCEKE GRPRMTQIE DLPDHEVNS GKFNPI.CKA SCHPLPTSEE MTLVKPDGTV
TEK K---H P-f---S-N-G-M---P---R- -I---K-V ---N---
lie --S-Q---A P-Hf-A-R- Q-Q-Q-CGT- ---S-V-PS -H-VH---S D-...I---L NMASEL-f-L ETMPR-N-A- A-N-f-VRGS IE-R---

401 450 500
lie-2 LOPNDFNYTD RFSVAIFTN RVLPPDSGW VCSNTVAGM VEKPFNISVK VLPEPLHAPN VIDTGHNAI INISSEPYFG DGPIKSKKLF YKPVN..QAW
TEK -H-K---H- H---IH -I---K-N---V ---L---HYE---
lie -LSTKAIVEP EKIT-EFE-P -LVLA---f- E-R-S-SG-Q DSRR-KVN- -P-V-A-R LL.-KQSRQL WSPLVSFS- ---STVR-H -R-QDSTMD-

FIG.3A

501 550 600
lie-2 KYIEV.TNEI FILNLEPRT DYELCVOLAR PGECEGHPG PVRFTTASI G.LPPRGLS LLPKQTALN LTHQIFTNS E...DEFYVEV ERRSLQTTSD
TEK OH-Q- V- E- V- R- N- T- PS- -D- V-K-
lie ST-V-DPS-N V-MN-R-K- G-SVR-S- AW- PTLM-DCP EP-LQ-WLEG WHVEGTDR-R VS-SLPLVPG PLVCDGFLLR LWDGTRQDER
601 650 700
lie-2 QONIKVPGNL TSVLLSNLVP REQYTVRARV NTKAQEWSE ELRAWTLSDI LPPQENIKI SNITDSTAMV SWTIVDGY.. SISSIIIRYK VQGKNEQCHI
TEK N-H- V- D-T- H-S-VI L-.. T- V-
lie RE-VSS-OAR -A.- IG-T- GTH-OLDVOL YHCTLLGPAS PPAHVL-PPS G-A-RHLHA QALS-EIQL T-KHPEALPG P-KYVVEVQ -A-GAG-P.L
701 750 800
lie-2 DVKIKNATVT QYQLKGLEPE ITYNDIFA.ENN IGSSNPAFVSH ELRTLPHSPA SADLGGGKML LIAILGSAGM TCITVLLAFL IMLQLKRAV
TEK II- A-Q- V-E-Q- P- L- -I-
lie WIDVORPEE- STIIR-NAS -R-LFRMR-S IQGLGOWS-T VEE-TLGNGL QAECPVQESR A-EE-LDQQ- IL-VV-VSA -L-I-A-L- TLVCIR-SCL
801 850 900
lie-2 QRRMAQAFON VR.EEPVQF NSGTLALNRK AKNINPDPTIY PVLWDNDIKF QDVIGEGNFG QVLKARIKKD GLRMDAAIKR MKEYASKDDH RDFAGELEVL
tek
TEK
lie H-RIFTY-S GSG-TIL- S-T-T-R P-LQ-E-LS- E-E-T- E-L- -IR-M- L-EN-
901 950 1000
lie-2 CKLGHHPNI I NLLGACEHRC YLYLAIEYAP HGNLDFLRK SRVLETOPAF AIANSTASTL SSQQLLHFAA DVARGMDYLS QKQF IHRDLA ARNILVGENY.
tek M-
TEK
lie KN- I- Y- REHC- R-R-S -A-N-Q- E- V-L

FIG.3B

FIG. 3C

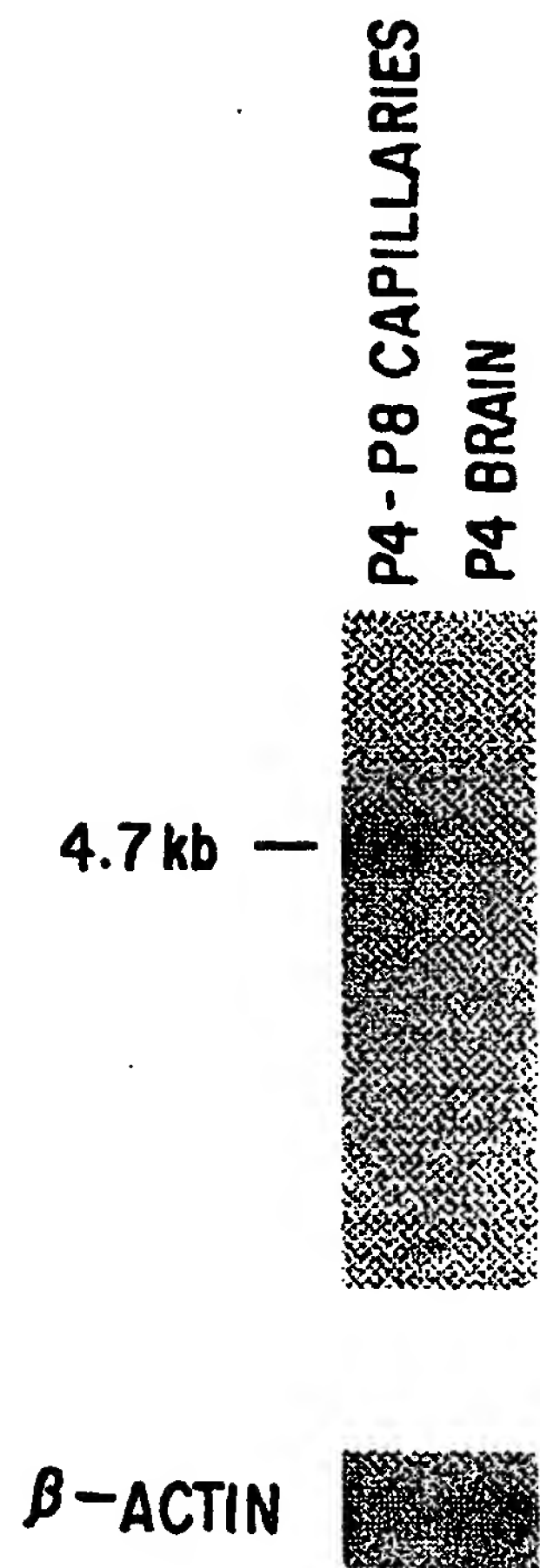


FIG. 4

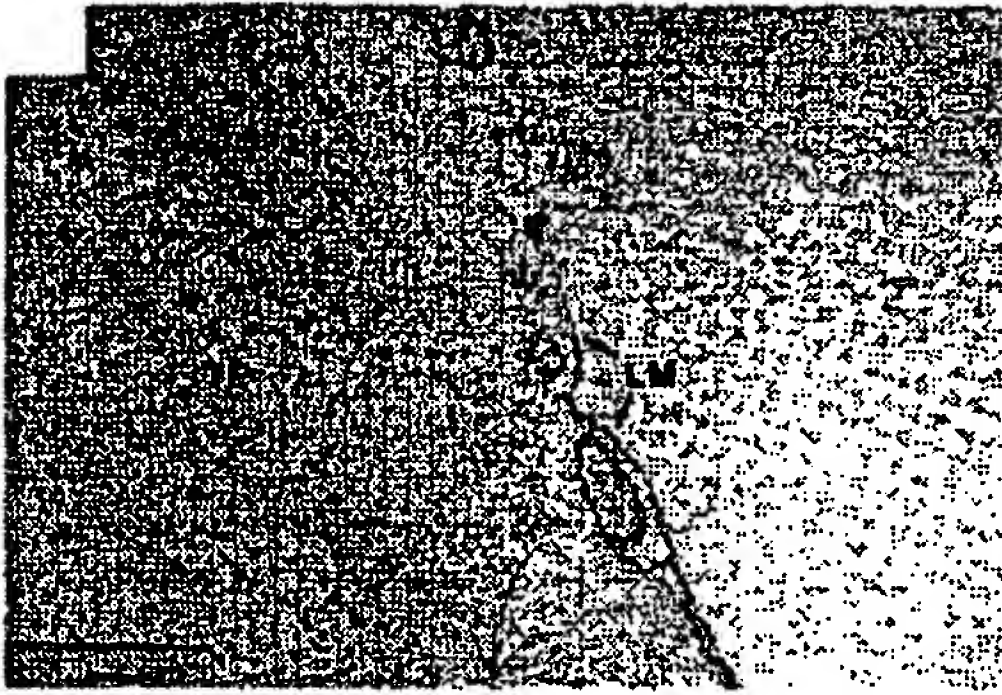


FIG. 5A

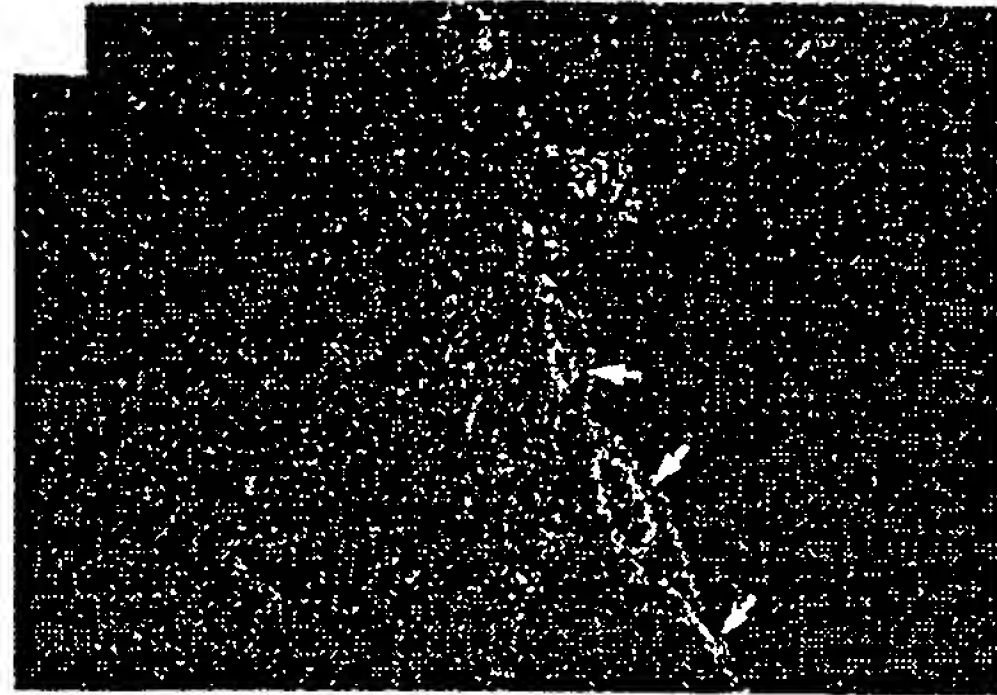


FIG. 5B

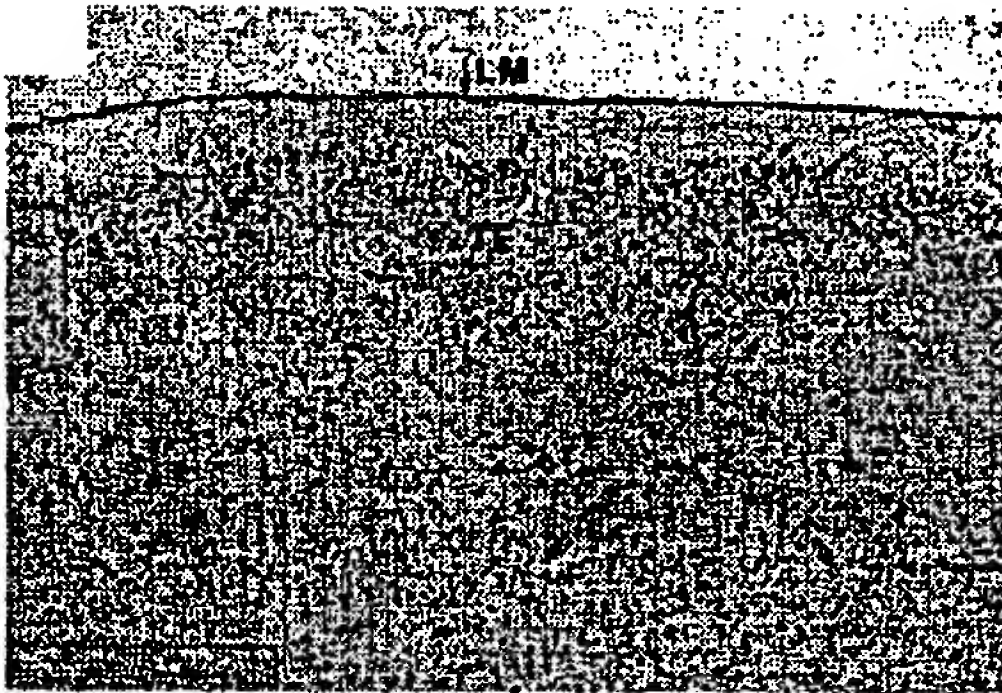


FIG. 5C

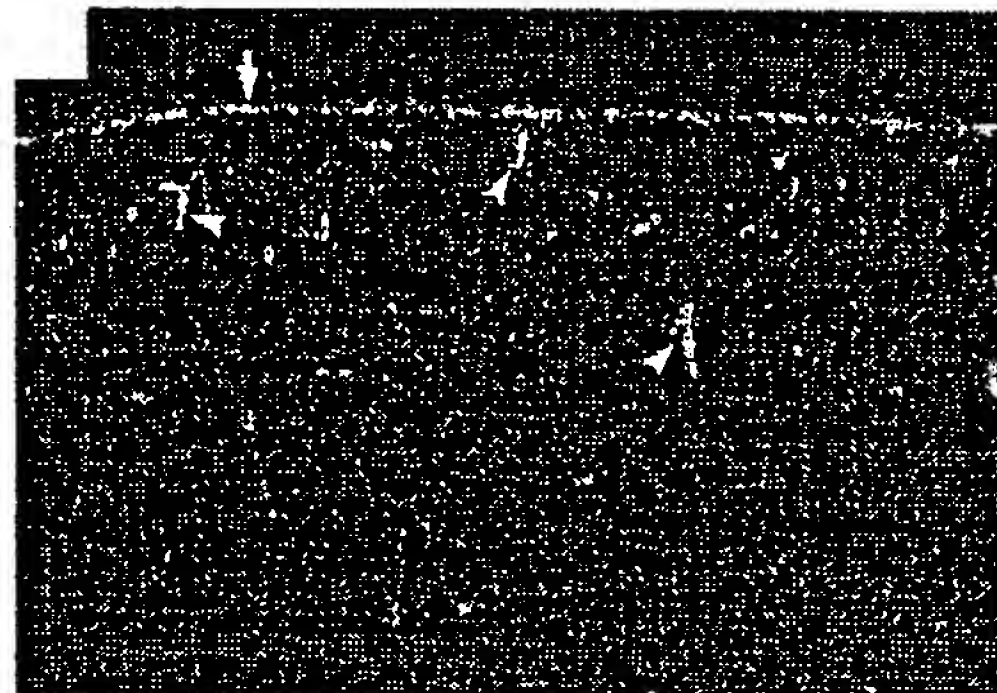


FIG. 5D



FIG. 5E

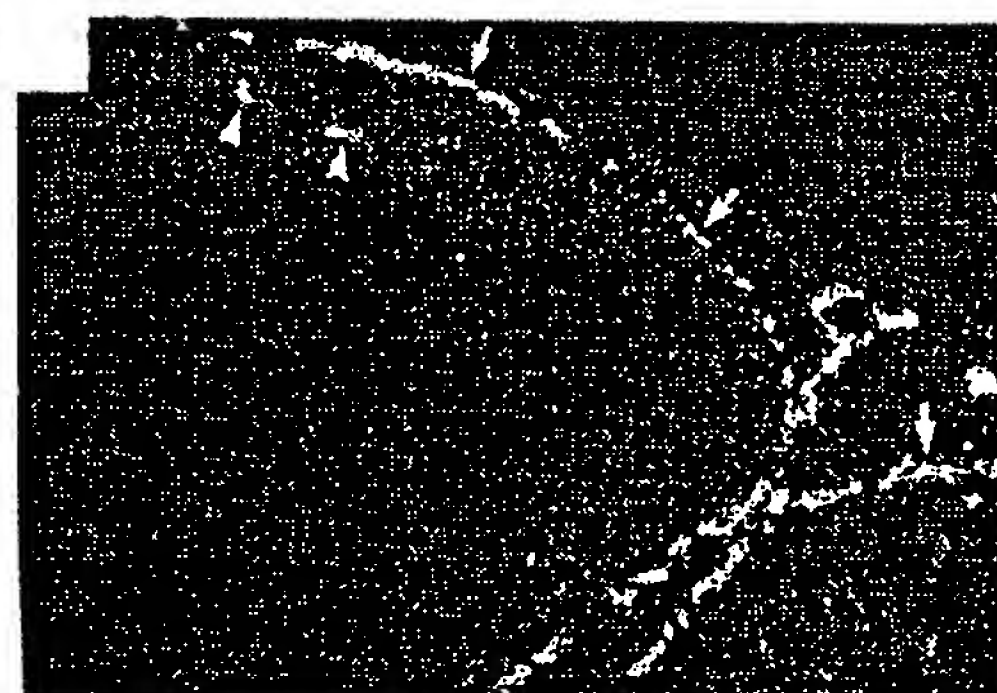


FIG. 5F



FIG.5G



FIG.5H

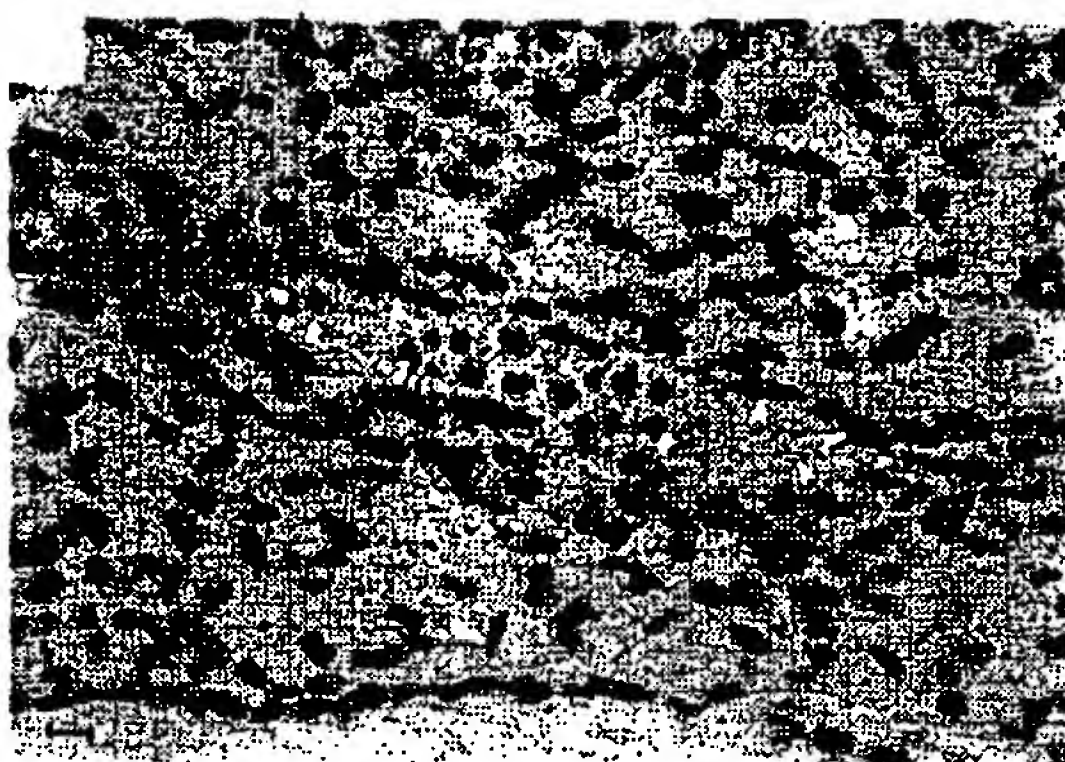


FIG.6A

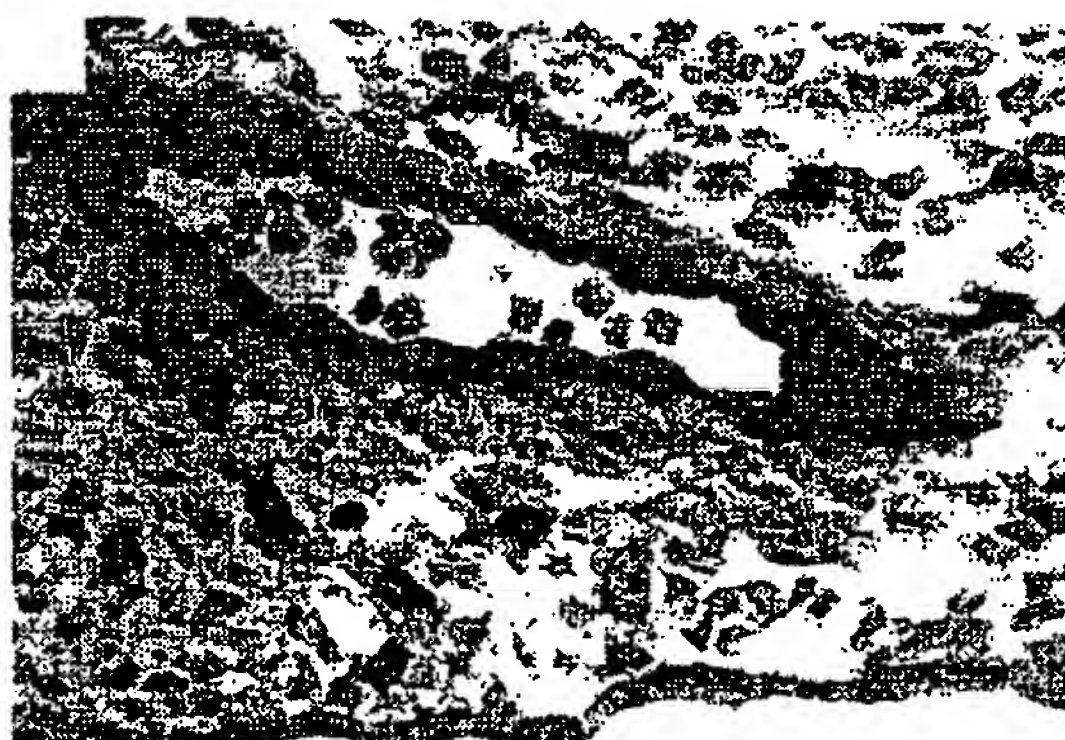


FIG.6B

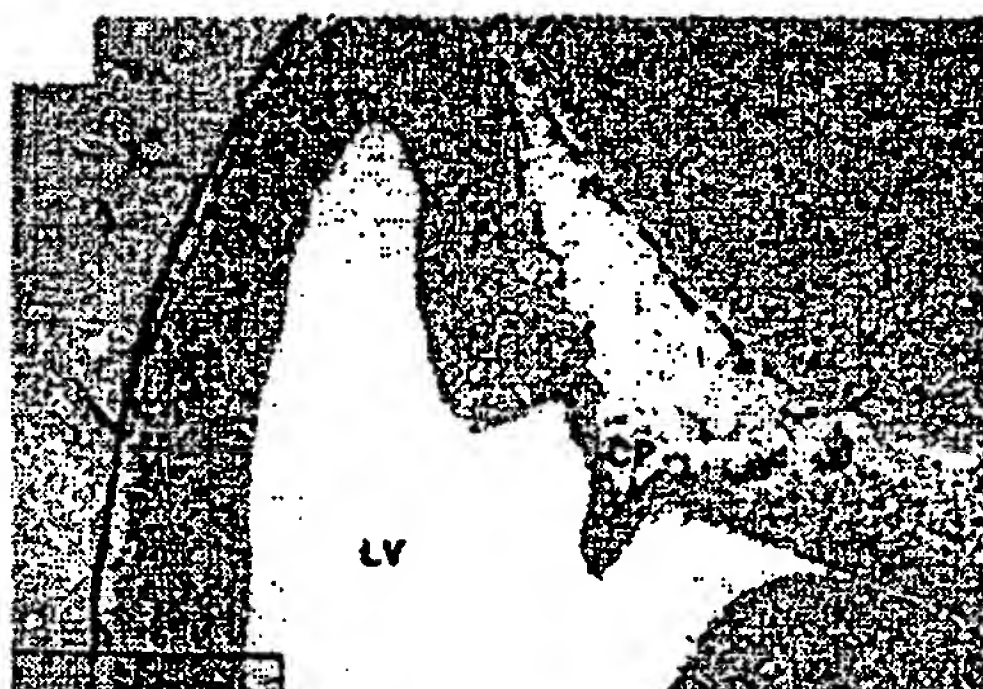


FIG. 7A

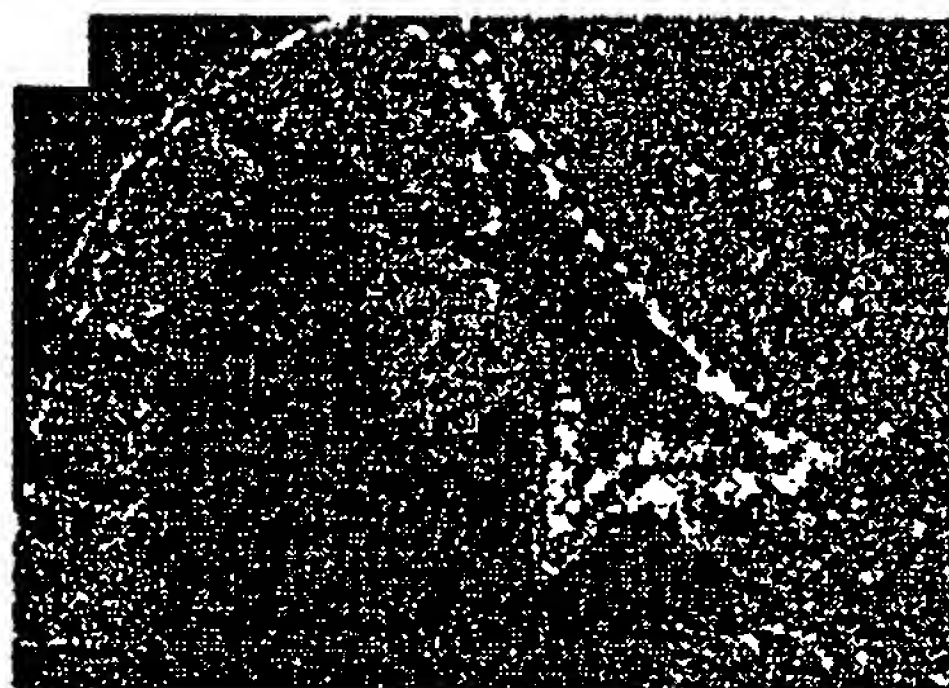


FIG. 7B

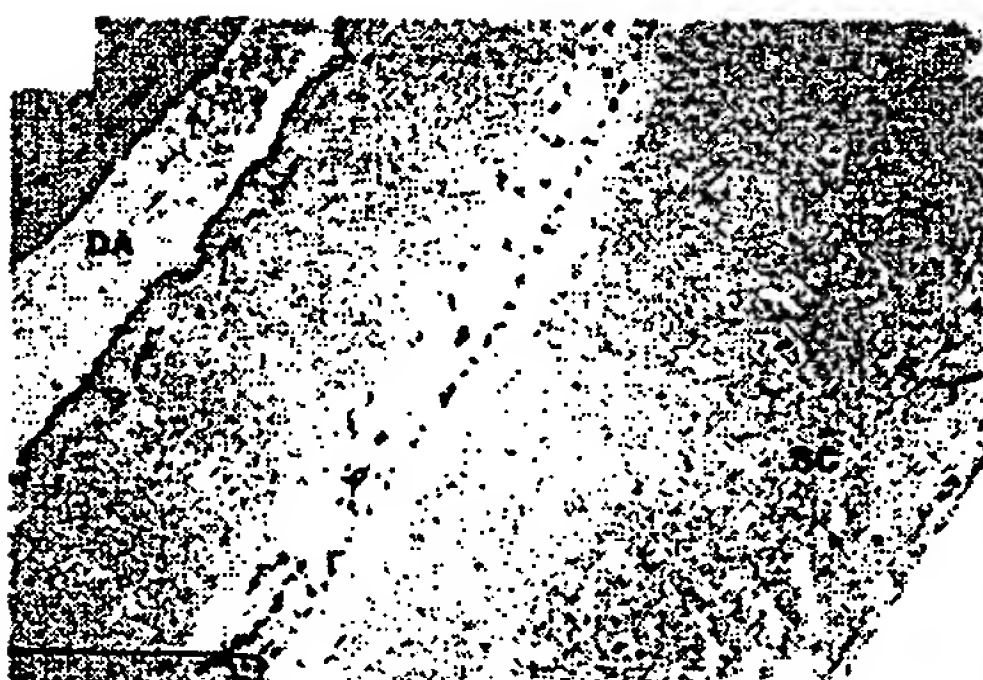


FIG. 7C

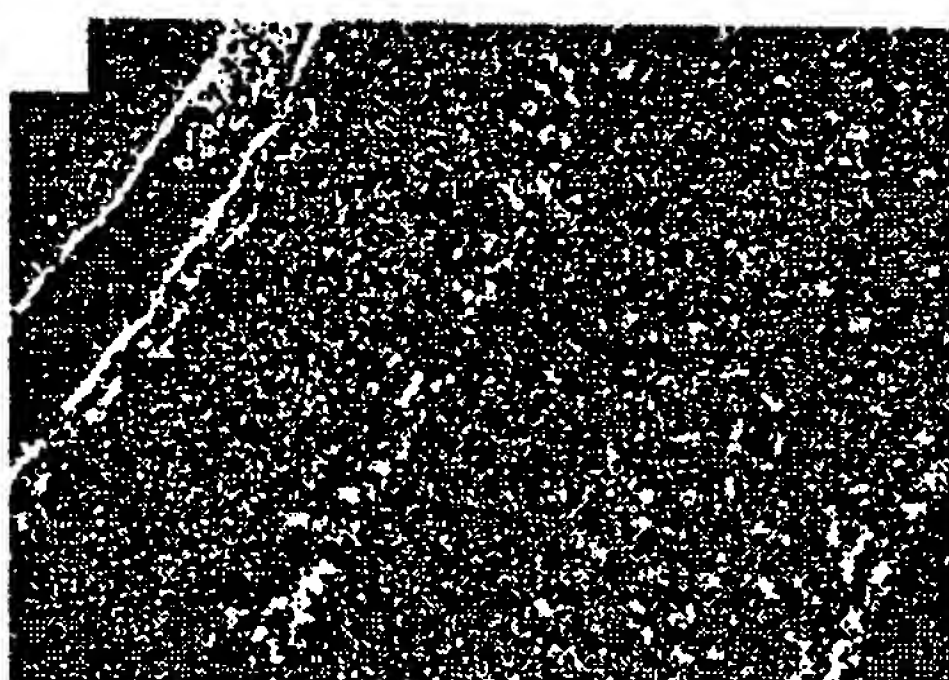


FIG. 7D



FIG. 7E



FIG. 7F



FIG. 7G

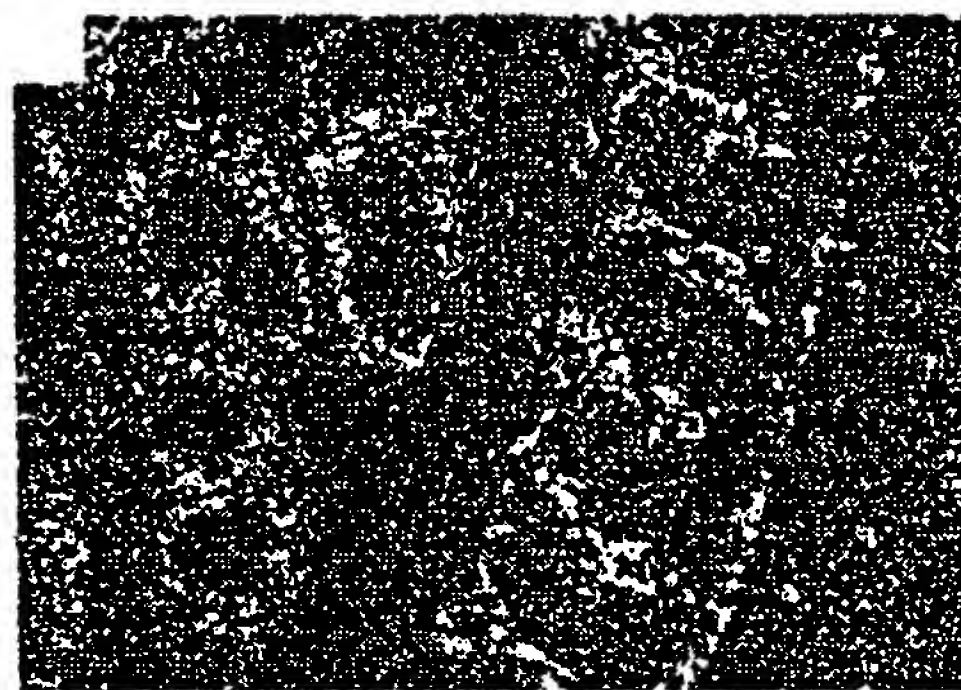


FIG. 7H



FIG.8A



FIG.8B



FIG.8C

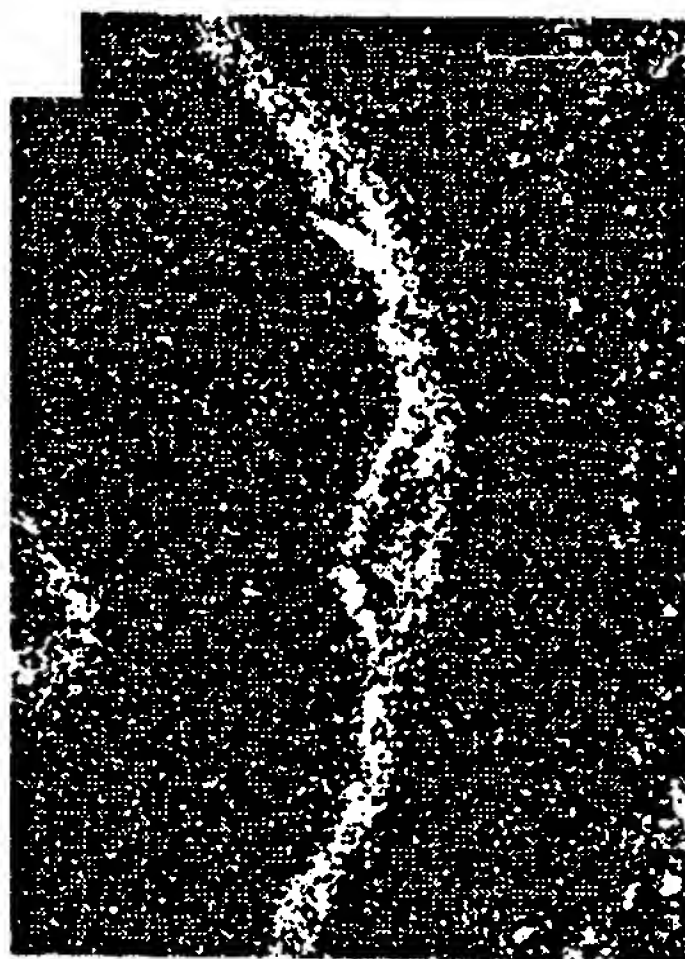


FIG.8D

Best Available Copy
INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/03767

A. CLASSIFICATION OF SUBJECT MATTER

**IPC 6 C12N15/63 C07K14/47 C07K14/515 C12N15/62 C12N5/16
C07K16/28**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DEVELOPMENT, vol. 119, 1993 pages 957-968, H. SCHNÜRCH ET AL. 'Expression of tie-2, a member of a novel family of receptor tyrosine kinases, in the endothelial cell lineage' *see the whole document* ---	1-38
X	GROWTH FACTORS, vol. 9, 1993 pages 99-105, A.S. RUNTING ET AL. 'tie-2, a putative protein tyrosine kinase from a new class of cell surface receptor' *see the whole document* --- -/--	1-38

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search

2 March 1995

Date of mailing of the international search report

04. 04. 95

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Authorized officer

Marie, A

INTERNATIONAL SEARCH REPORT

International Application No
PC1/EP 94/03767

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ONCOGENE, vol. 8, 1993 pages 1631-1637, P.C. MAISONPIERRE ET AL. 'Distinct rat genes with related profiles of expression define a TIE receptor tyrosine kinase family' *see the whole document* ---</p>	1-38
X	<p>PNAS, vol. 90, 1993 pages 9355-9358, T.N. SATO ET AL. 'tie-1 and tie-2 define another class of putative receptor tyrosine kinase genes expressed in early embryonic vascular system' *see the whole document* -----</p>	1-38